Chapter 5: Identification, Assay and Related Substances

Introduction

The BP, *Ph.Int.* and USP each has a monograph for the analysis of quinine sulfate tablets. The quantitative methods were successfully transferred/verified to ensure accurate and reliable results (Chapter 4). The purpose of this study was to critically evaluate the possible differences between the outcomes of the prescribed tests (if any). Four commercially available products of quinine sulfate tablets were used for this study (refer to Chapter 3, section 3.8). All analytical tests specified in the monographs pertaining to quinine sulfate tablets were executed (except where justified otherwise in the respective discussions that follow). This chapter describes the results obtained from identification, uniformity of dosage units, assay and related substances testing. Chapter 6 will elaborate on the API release testing (dissolution and disintegration) from the solid oral dosage form.

Table 5-1 provides a summary of tests discussed in Chapter 5.

Table 5-1: Analytical QC tests for the quinine sulfate tablet monographs of the USP, BP and *Ph.Int*. (pertaining to Chapter 5)

Test	Technique	USP	BP	Ph.Int.
ID	TLC	Yes	Yes	Yes
	HPLC	Yes	No	Yes
	UV	No	No	Yes
	Fluorescence	Yes	No	No
	pH	No	Yes	Yes
	Sulfate precipitation	Yes	Yes	Yes
Uniformity of dosage units	Uniformity of mass/Weight variation	Yes*	Yes*	Yes*
	Uniformity of content	No	No	No
Assay	HPLC	Yes	No	No
	Non-aqueous titration	No	Yes	Yes
Related substances (other cinchona alkaloids or chromatographic purity)	HPLC	No	Yes	Yes
	TLC	Yes	No	No

* general pharmacopoeial requirement for tablets. Not a specific procedure that applies to quinine sulfate tablets monograph, but all tablet monographs.

5.1 Identification

The purpose of identification tests are to establish the identity of an API in a product (Kotz *et al.,* 2003:14). This is usually done by means of a series of tests that could include physical constants, chromatographic behaviour and chemical reactivity. Physical constants include UV-Vis absorbance spectra, solubility, melting point and boiling point measurements (Kotz *et al*., 2003:14). The ICH Q6A guidelines specify that identification tests should discriminate between compounds that are similar and that identification by a single technique is not regarded as specific enough (ICH, 1999). It is therefore necessary to perform a combination of tests to obtain the required level of specificity. All three of the monographs employ a variety of identification tests for quinine sulfate tablets (summarised in Table 5-1) and are therefore in compliance with the ICH Q6A guidelines.

5.1.1 Fluorescence

The USP monograph describes the fluorescence test as part of a series of identification tests that need to be performed to ensure the accurate identification of quinine sulfate. Quinine is natively fluorescent and a positive outcome for this procedure indicate the presence of quinine in the sample solution (Watson, 2005:152). Please refer to the discussion in Chapter 3 (section 3.1.1) for the reasoning behind the suitability of this test as a means to identify quinine sulfate.

A quantity of the powdered tablets equivalent to 100 mg of quinine sulfate was dissolved and well shaken in 100 ml of diluted sulphuric acid (1 in 350). A vivid blue fluorescence (Figure 5-1) was observed when the sample was evaluated under UV light at 366 nm. The fluorescence disappeared when a few drops of hydrochloric acid was added to the solution and thus complied to the requirements of the monograph (Figure 5-1 and Table 5-2).

Figure 5-1: A typical example depicting the fluorescent ability of quinine sulfate in sulphuric acid solution (left) which is quenched by the addition of hydrochloric acid (right).

All four products were submitted to the fluorescence test described in the USP monograph. The results obtained are tabulated in Table 5-2.

Table 5-2: The outcomes of the fluorescence test

Product	A vivid blue fluorescence was observed when the tablet powder was dissolved in sulphuric acid	The fluorescence disappeared upon addition of a few drops of hydrochloric acid
1	Yes	Yes
2	Yes	Yes
3	Yes	Yes
4	Yes	Yes

5.1.2 Precipitation reactions

The ICH (Q6A) guidelines state that if an API is in the form of a salt, identification tests should test for the presence of the individual salt ions (ICH, 1999). For this reason the test for sulfates need to be performed for quinine sulfate. All the pharmacopoeia are in compliance with this requirement, although the test methodology between monographs differ slightly.

All three of the pharmacopoeia included the sulfate ion identification test in their quinine sulfate tablet monographs. The procedures to test for the presence of sulfates described by the three quinine sulfate monographs USP, BP and *Ph.Int.* are summarised in Table 5-3.

Table 5-3: A summary of the procedures for the test for sulfates utilised by the various pharmacopoeia

	BP	USP	Ph.Int.
Step 1	Extract a quantity of powdered tablet containing 0.1 g of quinine sulfate with 20 ml water and filter.	Extract a quantity of powdered tablet containing 20 mg of quinine sulfate with 10 ml of dilute hydrochloric acid and filter.	Extract a quantity of powdered tablet containing 0.1 g of quinine sulfate with 10 ml water and filter.
Step 2	Add 1 ml of hydrochloric acid and 1 ml of barium chloride to 5 ml of the solution obtained in step 1 - a white precipitate should form.	Add barium chloride to the solution obtained from step 1 - a white precipitate should form which should be insoluble in hydrochloric acid and nitric acid.	Add barium chloride to the solution obtained from step 1 - a white precipitate should form which should be insoluble in hydrochloric acid.
Step 3	Add 0.1 ml 0.05 M iodine to the suspension obtained from step 2 - the suspension should turn into a yellowish colour. When stannous chloride is added dropwise to this solution, the suspension should be decolourised. When the mixture is boiled no coloured precipitate should form.	Addition of lead acetate to the solution obtained in step 1 will form a white precipitate which is soluble in ammonium acetate.	

The suitability and reasoning behind all the different steps of the precipitation tests have been discussed in Chapter 3, section 3.1.2.

The first two steps to test for the presence of sulfates is to extract quinine sulfate either with water (BP and *Ph.Int.* monographs*)* or diluted hydrochloric acid (USP) and thereafter react it with barium chloride to form a white precipitate of barium sulfate ($BaSO_4$), which is practically insoluble in water and in acid (Skoog, 1997:94 and Kotz *et al.,* 2003:155). The USP also specifies that when quinine sulfate is dissolved in the hydrochloric acid, no precipitation should form.

After step 2, the BP and USP monographs each have slightly different approaches for the sulfate test, whereas the *Ph.Int.* monograph do not provide any further steps.

In addition to the mutual steps (step 1 and 2), the BP monograph further require (step 3) the addition of 0.05 M iodine solution to the $BaSO_4$ suspension which must turn the suspension yellowish/brown and thereafter the addition of stannous chloride to decolourise the suspension (explained in Chapter 3, section 3.1.2). The mixture must then be boiled for ± 5 - 10 minutes not allowing the precipitate to dissolve. In order for the test to comply, all the changes described above must be met. Figure 5-2 depicts the sulfate test of the BP quinine sulfate tablet monograph.

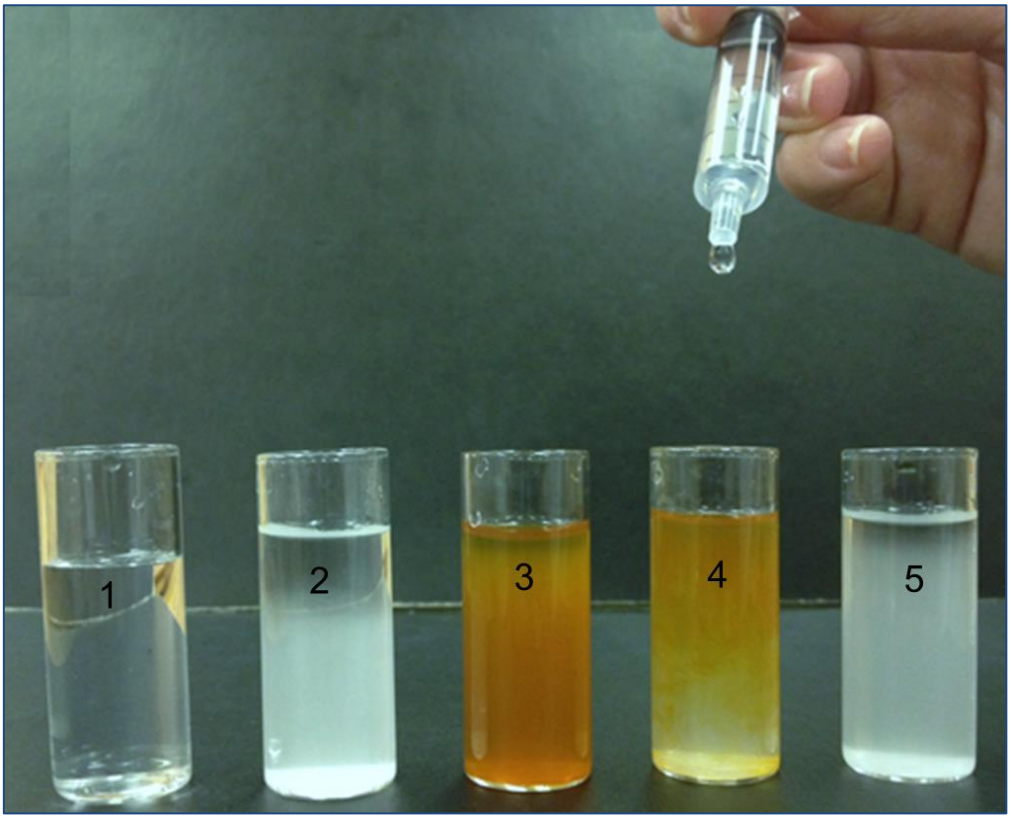

Figure 5-2: **An example of the test for sulfates employed by the BP. A clear quinine sulfate aqueous solution (1) formed a white precipitate (2) when the barium chloride was added, a yellow/brown suspension when iodine was added (3) and when stannous chloride was added dropwise (4) to the solution it decolourised to the milky suspension again (5).**

In addition to the mutual steps (steps 1 and 2), the USP require that lead acetate be added to an additional quinine sulfate solution obtained from step 1 (not the $BaSO_4$ precipitate solution). A white precipitation must form, which must dissolve when ammonium acetate is added to it (step 3). The USP also specifies that when quinine sulfate is dissolved in the hydrochloric acid, no precipitation should form.

All samples were subjected to each procedure and specification(s) of the different monographs. All the samples tested positive for sulfates (Table 5-4).

The *Ph.Int.* monograph list two individual sulfate tests (similar to the USP - refer to step 2 and 3) in its general chapters, but its quinine sulfate monograph requires only that test A be executed. As mentioned previously a number of identification tests that are combined, improve the specificity of identification. One could thus conclude from this that the *Ph.Int.* monograph for quinine sulfate tablets might be somewhat less specific to positively identify the presence of sulfate (performing only test A) in comparison with the BP and USP monographs. Fortunately, the *Ph.Int.* monograph also requires a number of other identification tests to be performed, which improve specificity – therefore there is no concern at this point that the identification tests of the *Ph.Int.* monograph are inadequate.

Table 5-4: Outcomes for the test for sulfates

Product	1	2	3	4
BP	Comply	Comply	Comply	Comply
USP	Comply	Comply	Comply	Comply
Ph.Int.	Comply	Comply	Comply	Comply

5.1.3 Measuring pH

The BP and the *Ph.Int.* monograph requires as part of a series of identification tests, that the pH of a 10 mg/ml quinine sulfate suspension be measured. The procedures specified are compared in Table 5-5. The differences between the two methods are presented in **bold.**

Table 5-5: Identification by means of pH utilised by the BP and *Ph.Int.*

	BP	***Ph.Int*.**
Step 1	A quantity of tablet powder equivalent to 250 mg quinine sulfate is extracted with 25 ml of a solution consisting of 2 volumes of chloroform and 1 volume of ethanol (96%) and thereafter filtered.	
Step 2	The filtrate is evaporated to dryness (example shown in Figure 5-3).	
Step 3	The residue is then washed with 10 ml of ether, filtered and again washed with another 10 ml of ether.	
Step 4	The residue is thereafter dried in a **vacuum oven** at a pressure not exceeding 15 Pa and at 60°C for **2 hours.**	The residue is thereafter dried in an **oven** at 60°C for **5 hours**.
Step 5	The dried residue is used to prepare 10 mg/ml aqueous suspension using **carbon dioxide free water.**	The dried residue is used to prepare 10 mg/ml aqueous suspension using **purified water.**
Specifications	pH between **5.7** and **6.6**	pH between **5.5** and **7.0**
Average of specification (± deviation from average)	6.15 ± 0.45	6.25 ± 0.75

From Table 5-5 it can be seen that steps 1 - 3 are communal between monographs but that steps 4 and 5 are different between the BP and *Ph.Int.* monographs. One can also derive from Table 5-5 that the monographs allow different deviations from the average (theoretical anticipated pH). The *Ph.Int*. monograph allows a deviation of 0.75 pH units from the average, whereas the BP monograph allows only 0.45 pH units from the average.

At first it seems as if the BP monograph procedure employs stricter specifications in comparison with the *Ph.Int.* monograph, however these differences are not unjustified. The difference in the allowed variance is justified by the fact that the *Ph.Int.* monograph procedure needs to take into consideration the day-to-day fluctuations in the pH of water, whereas the BP monograph procedure need not, as it specifies the grade of water (carbon dioxide free). It is well known that the pH of water is influenced by the amount of carbon dioxide dissolved in the water, which can cause day-to-day fluctuations in the pH of water.

Figure 5-3: The residue that formed after the solutions were evaporated to dryness (Product 1, (1), Product 2 (2), Product 3 (3) and Product 4 (4)).

The samples of the four products were prepared according to the procedures of both monographs. The results showed compliance with their respective, corresponding specifications, however the result sets obtained for the same product differed (refer to Table 5-6 and Figure 5-3). The influence of carbon dioxide in the different types of water was evident, which exemplified the justification of specification differences between the two monographs.

Table 5-6: The pH of the quinine sulfate suspensions according to the test procedures of the *Ph.Int.* and BP

Product	*Ph.Int.*	BP	Complies	
			Ph.Int.	BP
1				
Sample 1	6.8	6.6	Yes	Yes
Sample 2	6.9	6.5		
Sample 3	6.9	6.6		
Average	**6.8**	**6.6**		
%RSD	**0.08**	**0.49**		
2				
Sample 1	6.9	6.6	Yes	Yes
Sample 2	6.9	6.6		
Sample 3	6.9	6.6		
Average	**6.8**	**6.6**		
%RSD	**0.15**	**0.09**		
3				
Sample 1	6.8	6.6	Yes	Yes
Sample 2	6.8	6.6		
Sample 3	6.8	6.6		
Average	**6.9**	**6.6**		
%RSD	**0.08**	**0.15**		
4				
Sample 1	7.0	6.6	Yes	Yes
Sample 2	7.0	6.6		
Sample 3	7.0	6.6		
Average	**7.0**	**6.6**		
%RSD	**0.08**	**0.09**		

5.1.4 Ultraviolet spectrophotometry

The *Ph.Int.* monograph requires an identification test for quinine sulfate by utilising UV-Vis spectrophotometry. 50 ml of 0.1 M hydrochloric acid was added to tablet powder equivalent to 15 mg of quinine sulfate. The solution was filtered and 5 ml of the filtrate was diluted to 50 ml with 0.1 M hydrochloric acid. The specifications of the monograph stated that the absorbance maxima should be approximately at 347 nm (345 - 349 nm). The solution was scanned using a Cary 50 UV-Vis spectrophotometer (from 200 nm to 800 nm). An absorbance maximum was observed at 348 nm for all the samples. Figure 5-4 shows the UV-Vis absorption spectra of the four samples, indicating the absorption maxima at 348 nm. All four products comply to the test specifications (Table 5-7), as a deviation of 2 nm is allowed from the specified absorbance maxima (BP, 2013).

Table 5-7: Outcome for the UV-Vis identification test

	Product 1	**Product 2**	**Product 3**	**Product 4**
Abs peaks at:	318 nm 348 nm	318 nm 348 nm	318 nm 348 nm	318 nm 348nm
Abs max at:	348 nm	348 nm	348 nm	348 nm
Ph.Int. monograph	Comply			

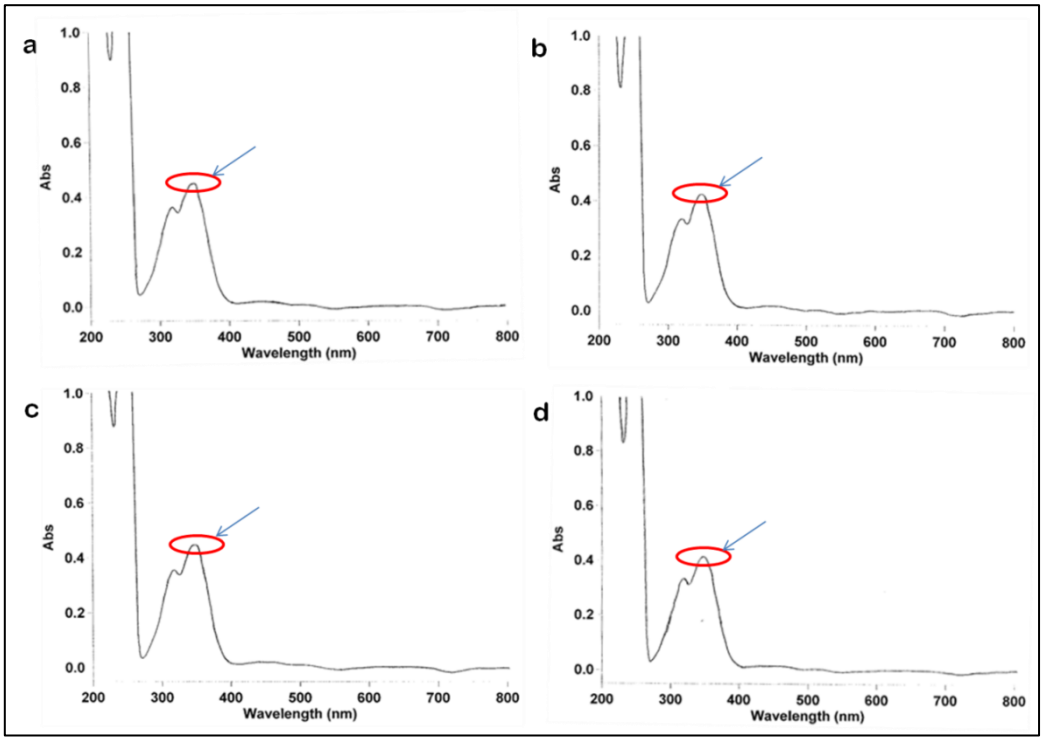

Figure 5-4: The UV absorbance spectra of the four different products a) Product 1, b) Product 2, c) Product 3 and d) Product 4

5.1.5 Thin Layer Chromatography (TLC)

The BP and *Ph.Int.* monographs describe a TLC method for the identification of quinine sulfate whereas the USP monograph describe a TLC method that is used both for identification and impurity testing. The *Ph.Int.* monograph describes two different TLC tests (A.1 and A.2) for the identification of quinine sulfate. Test A.2 is similar (with only minor differences) to the TLC method of the BP monograph. The *Ph.Int.* monograph further provides a choice between A.1 and A.2, where A.2 should be performed only when UV detection is not available. Duly, only test A.1 of the *Ph.Int.* monograph was performed, seeing that UV detection was available. Section 5.1.5.1 to 5.1.5.3 describe the chromatographic conditions, mobile phase and procedures for identification by means of TLC.

5.1.5.1 The BP monograph TLC method:

TLC plate:	Silica gel R6
Mobile phase:	10 volumes diethylamine
	20 volumes acetone
	80 volumes toluene

Solvent:	2 volumes chloroform 1 volume ethanol (96%)
Solution 1:	Transfer powdered tablet containing 100 mg of quinine sulfate into a 10 ml volumetric flask. Make up to volume with solvent, mechanically shake and filter.
Solution 2:	Contains 1% w/v quinine sulfate in solvent.
Solution 3:	Contains 1% w/v quinidine sulfate and 1% w/v quinine sulfate in solvent.
Application:	2 µl of each solution and allow the mobile phase front to move 15 cm.
Detection:	Allow the plate to dry in air and spray with 0.05 M ethanolic sulfuric acid and then with dilute potassium iodobismuthate solution.
Specifications:	The principal spot in the chromatogram obtained with solution 1 corresponds to that in the chromatogram obtained with solution 2. The test is not valid unless the chromatogram obtained with solution 3 shows two clearly separated spots.

Figure 5-5 depicts the process that was followed for the identification of quinine sulfate using the TLC identification test of the BP monograph. The quinine and quinidine spots were clearly separated (solution 3) and the principal spot of the sample (solution 1) corresponded in appearance, position and intensity with that of the quinine sulfate standard (solution 2), complying with the specifications for identification (Table 5-8 and Figure 5-6).

Figure 5-5: **Demonstration (from left to right) of how the TLC plate was spotted, developed in a chromatographic chamber and sprayed for detection.**

Table 5-8: **R_f-values of the quinine sulfate spots present in standard and sample solutions for identification purposes - BP monograph**

	Standard solution 2	Product 1	Product 2	Product 3	Product 4
R_f value	0.16	0.15	0.16	0.16	0.16

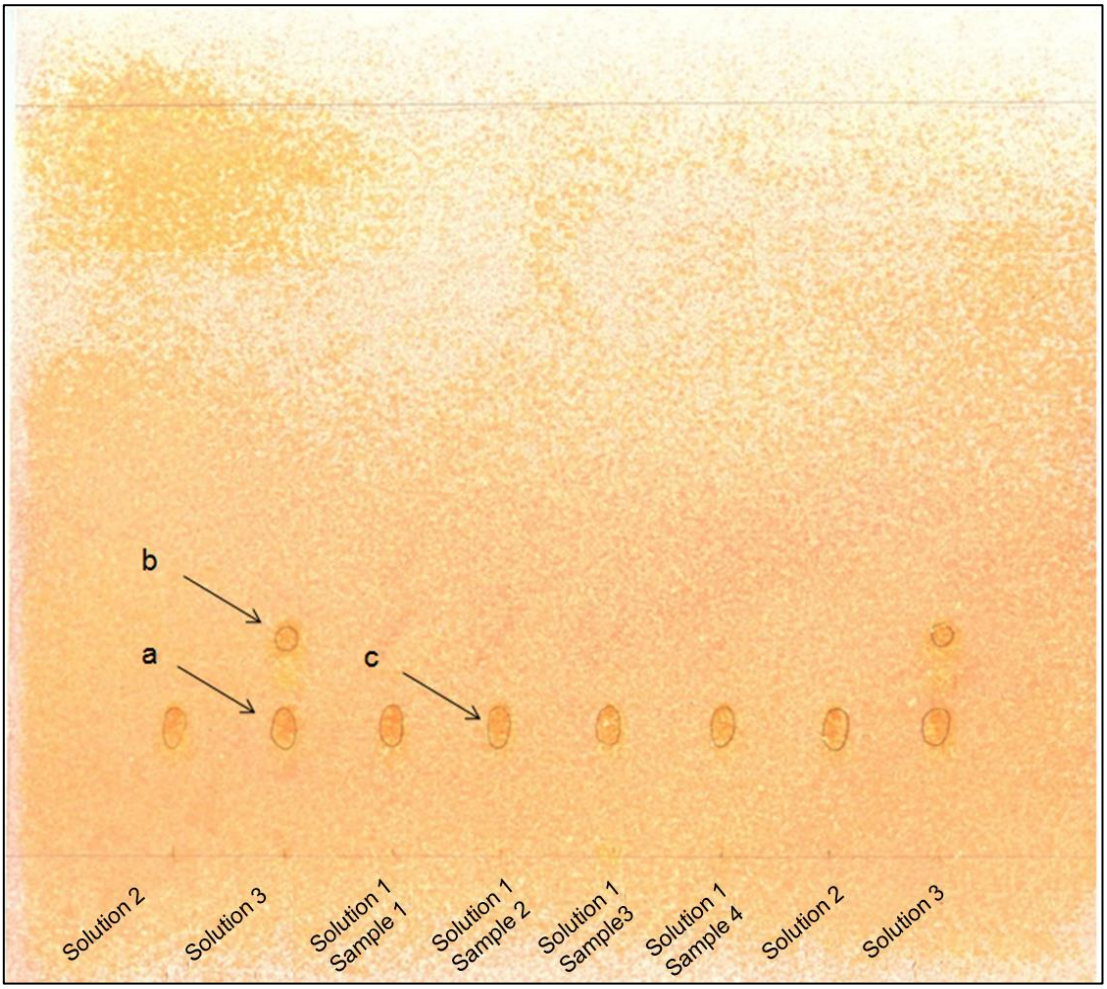

Figure 5-6: **TLC plate where (a) represents the spot for quinine sulfate, (b) represents the spot for quinidine sulfate and (c) represents the spot for quinine sulfate present in the products - BP monograph**

5.1.5.2 The *Ph.Int.* monograph TLC method (A.1):

TLC plate: Silica gel R6

Mobile phase: 5 volumes diethylamine
12 volumes ether
20 volumes toluene

Solvent: 2 volumes chloroform
1 volume ethanol (96%)

Solution A:	Transfer powdered tablet containing 100 mg of quinine sulfate into a 10 ml volumetric flask. Make up to volume with solvent, mechanically shake and filter.
Solution B:	Contains 1% w/v quinine sulfate.
Solution C:	Contains 1% w/v quinidine sulfate in solution B.
Application:	2 µl each of solution and allow the mobile phase front to move 15 cm.
Detection:	Allow the plate to dry in cool air and examine under a UV light (254 nm).
Specifications:	Solution C should show two separate spots and the principal spot obtained with solution A should correspond in position, appearance and intensity with that of solution B.

As seen in Figure 5-7, the quinine sulfate and quinidine spots (from solution C) were clearly separated, which complied with the system suitability requirements of the monograph. The spots obtained from the sample solutions (solution A) corresponded to that of the quinine sulfate standard (solution B), which ensured a positive identification for quinine sulfate (Table 5-9).

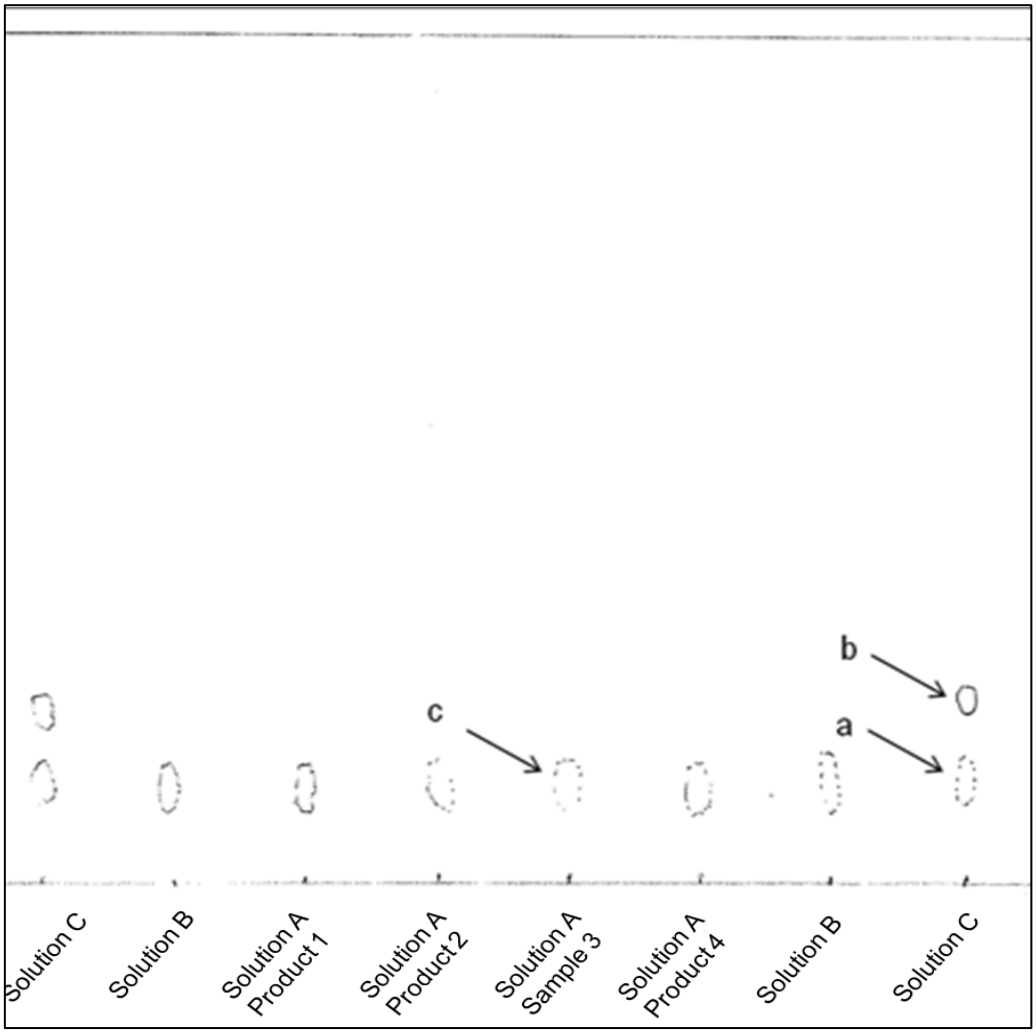

Figure 5-7: **TLC plate where (a) represents the spot for quinine sulfate, (b) represents the spot for quinidine sulfate and (c) represents the spot for quinine sulfate present in the various samples of the products using the *Ph.Int*. method.**

Table 5-9: **R_f-values of the quinine sulfate spots present in standard and sample solutions for identification purposes - *Ph.Int.* monograph**

	Standard solution 2	Product 1	Product 2	Product 3	Product 4
R_f value	0.11	0.11	0.11	0.11	0.11

The differences in the R_f-values observed for the BP method (Table 5-8) and *Ph.Int.* method (Table 5-9) could be attributed to the differences in the mobile phase composition of the mentioned methods.

5.1.5.3 The USP monograph TLC method:

TLC plate:	TLC plate with 0.25 mm chromatographic silica gel mixture as adsorbent
Mobile phase:	50 volumes chloroform 40 volumes acetone 10 volumes diethylamide
Solvent:	Diluted alcohol (C_2H_5OH)
Sample solution:	Transfer tablet powder equivalent to 150 mg of quinine sulfate into a 25 ml volumetric flask. Make up to volume with solvent, mechanically shake for 10 minutes and filter.
Standard solution A:	Contains 0.06 mg/ml quinine sulfate in solvent.
Standard solution B:	Contains 0.05 mg/ml of quininone RS (corresponding to 0.06 mg/ml of the sulfate) and 0.10 mg/ml of cinchonidine (corresponding to 0.12 mg/ml of the sulfate) in solvent.
Application:	10 µl each of the sample solution, solution A and solution B and allow the mobile phase front to move 15 cm.
Detection:	Allow the plate to dry in air for 5 minutes. Spray with glacial acetic acid and examine under long-wavelength UV light. Then spray the plate with potassium iodoplatinate TS.
Specifications:	For identification purposes the spot (R_f-value) produced by standard solution A (quinine) should correspond with R_f-value obtained from the spot of the sample solution.

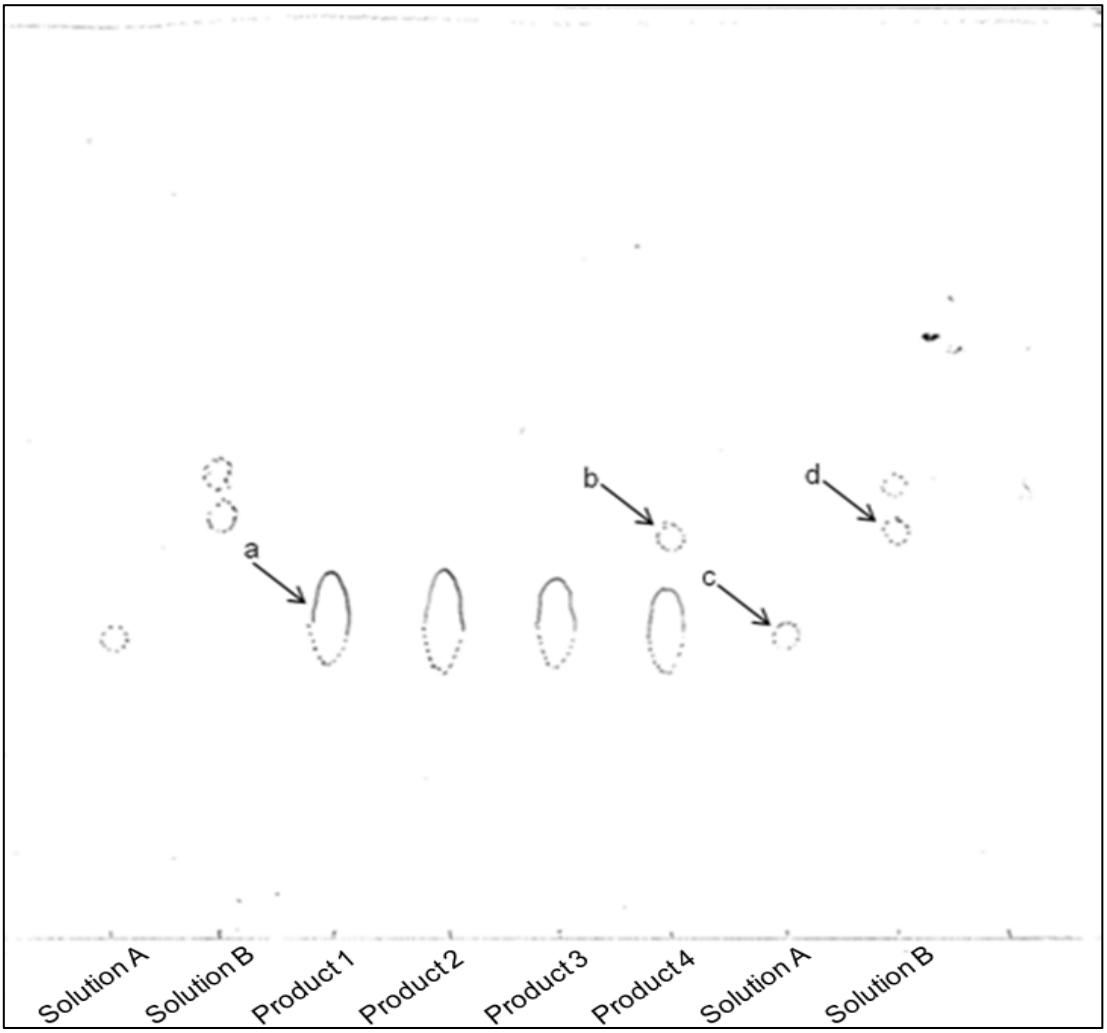

Figure 5-8: **TLC plate after being sprayed with glacial acetic acid, where (a) represents the spot for quinine present in the samples of the products, (b) represents the spot for cinchonidine present in the products, (c) represents the spot of the quinine present in solution A and (d) represents the spot for cinchonidine in solution B - USP method.**

As seen in Figure 5-8 the principal spots of the sample solutions corresponded to that of the standard solution A and thus demonstrated a positive identification for quinine. The quinine spots from the standard solution was less intense than that of the sample solutions, since the quinine sulfate concentration (0.06 mg/ml quinine sulfate) is less than the sample solution (6 mg/ml quinine sulfate) - refer to sample and standard preparations above. The difference in the spot intensity of quinine sulfate is justified by the difference in concentration. The concentrations are different between the sample and standard solution, since the test is also used for related substances/impurity determination – which is discussed in section 5.4.2 (refer to sample and standard preparations above). In Table 5-10 the calculated R_f-values for the samples (with

reference to quinine sulfate) also corresponded to that of the standard solution and complied to the identification specifications of quinine sulfate.

Table 5-10: R_f-values of the quinine sulfate spots present in standard and sample solutions for identification purposes

	Standard solution A	Sample 1	Sample 2	Sample 3	Sample 4
R_f value	0.32	0.34	0.34	0.33	0.32

Although the TLC identification methods of the different pharmacopoeia differ from each other, the same outcome (i.e. positive outcome for quinine sulfate) was obtained. Quinine sulfate was positively identified in each of these methods. All methods adequately provide requirements for specificity between similar compounds (adequate separation between spots was obtained).

5.1.6 High performance chromatography (HPLC)

Identification by means of HPLC is discussed in conjunction with assay/related substances by means of HPLC – see section 5.3.

5.2 Consistency of API content in dosage units

As explained in Chapter 3 (section 3.6), the consistency of the content of API dosage units are evaluated by means of uniformity of mass (UoM) or uniformity of dosage units testing. The *Ph.Int.* and BP monographs for quinine sulfate tablets have no specific procedure provided for dosage uniformity testing. As mentioned in Chapter 3 (section 3.6), should the specific product monograph (in this case) not provide with a specific method for dosage uniformity, then the general monograph requirements for tablets (oral solid dosage forms) apply (Figure 3-13, Chapter 3, section 3.6). Products 1 - 4 contained quinine sulfate (API) exceeding 25 mg/25% or more of the total weight (requirements of BP/USP monograph) and 5 mg/5% or more of the total weight (requirements of *Ph.Int.* monograph). Therefore weight variation (WV) had to be done in accordance with BP/USP requirements/methodology, whereas uniformity of mass (UoM) testing had to be done in accordance with *Ph.Int.* monograph requirements/methodology.

The USP monograph for quinine sulfate tablets presents with a specific quantitative UV method for content uniformity. Despite the specific procedure, reference is also made to <905>, the general chapter on uniformity of dosage units. This general chapter states the criteria and requirements for uniformity of dosage units. The criteria and requirements of the <905> is identical to the BP. Therefore for all the products pertaining to this study (Products 1-4) weight

variation testing was applicable. Should the products of this study have fallen under the criteria of content uniformity testing (API < 25 mg and/or < 25% of tablet mass), then the specific procedure of the quinine sulfate tablets monograph would have applied. Since the specific procedure did not apply to this study, the method verification of the specific quantification method (content uniformity) test of the USP was not performed.

UoM methodology requires that 20 tablets be weighed and the average mass calculated. A minimum of 18 tablets should not deviate from the average mass by more than 5% and a maximum of 2 tablets should not deviate from the average mass by more than 10%. All four products met the requirements for the uniformity of mass test of the *Ph.Int*. monograph.

Table 5-11: Uniformity of mass results according to the *Ph.Int.* general requirements

Tablet	Product 1 (% deviation from average)	Product 2 (% deviation from average)	Product 3 (% deviation from average)	Product 4 (% deviation from average)
1	521.09 (1.6)	524.49 (0.1)	558.03 (0.8)	411.39 (0.6)
2	540.95 (2.1)	523.80 (0.1)	566.90 (0.8)	410.57 (0.4)
3	526.71 (0.6)	516.33 (1.5)	574.86 (2.2)	400.85 (2.0)
4	529.10 (0.1)	518.07 (1.2)	567.16 (0.8)	411.19 (0.5)
5	524.67 (1.0)	529.21 (1.0)	561.19 (0.2)	407.32 (0.4)
6	528.66 (0.2)	530.89 (1.3)	558.00 (0.8)	403.38 (1.4)
7	525.71 (0.8)	525.06 (0.2)	565.02 (0.4)	412.08 (0.7)
8	531.03 (0.2)	524.23 (0.0)	565.38 (0.5)	420.45 (2.8)
9	525.26 (0.9)	532.35 (1.6)	557.74 (0.9)	404.48 (1.1)
10	530.14 (0.1)	526.17 (0.4)	568.94 (1.1)	399.49 (2.4)
11	534.78 (0.9)	525.33 (0.2)	558.53 (0.7)	414.45 (1.3)
12	530.22 (0.1)	519.54 (0.9)	558.48 (0.7)	409.68 (0.1)
13	534.50 (0.9)	525.12 (0.2)	563.55 (0.2)	402.36 (1.7)
14	530.32 (0.1)	523.58 (0.1)	557.33 (0.9)	412.52 (0.8)
15	528.15 (0.3)	528.07 (0.8)	562.67 (0.0)	401.92 (1.8)
16	536.58 (1.3)	519.90 (0.8)	561.00 (0.3)	422.20 (3.2)
17	530.09 (0.1)	516.05 (1.5)	564.16 (0.3)	413.23 (1.0)
18	532.57 (0.5)	523.43 (0.1)	559.89 (0.5)	402.37 (1.7)
19	523.44 (1.2)	521.20 (0.6)	564.81 (0.4)	412.23 (0.8)
20	532.25 (0.5)	529.77 (1.1)	557.43 (0.9)	410.30 (0.3)
Minimum	521.09	516.05	557.33	399.49
Maximum	540.95	532.35	574.86	422.20
Range of mass (mg) (max - min)	19.86	16.30	17.53	22.71
Average	529.81	524.13	562.55	409.12
%RSD	0.89	0.88	0.83	1.54
Number of individual masses deviating from the average by more than 5%	0	0	0	0

Tablet	Product 1 (% deviation from average)	Product 2 (% deviation from average)	Product 3 (% deviation from average)	Product 4 (% deviation from average)
Number of individual masses deviating from the average by more than 10%	0	0	0	0

10 tablets were weighed in accordance with the BP/USP monograph procedure and the acceptance value was calculated using equation 3.9 in Chapter 3. The content is expressed as percentage of the label claim using the weight of the individual tablet and the average value of the assay.

The acceptance value (AV) must be less than 15. All four products met the requirements for the weight variation test (Table 5-12).

Table 5-12: Weight variation results according to the BP general requirements for consistency of dosage units

Tablet	Product 1 (mean of individual contents expressed as % of label claim)	Product 2 (mean of individual contents expressed as % of label claim)	Product 3 (mean of individual contents expressed as % of label claim)	Product 4 (mean of individual contents expressed as % of label claim)
1 2 3 4 5 6 7 8 9 10	521.09 (99.06%) 540.95 (102.84%) 526.71 (100.13%) 529.10 (100.59%) 524.67 (99.74%) 528.66 (100.50%) 525.71 (99.94%) 531.03 (100.95%) 525.26 (99.86%) 530.14 (100.78%)	524.49 (100.20%) 523.80 (100.07%) 516.33 (98.64%) 518.07 (98.97%) 529.21 (101.10%) 530.89 (101.42%) 525.06 (100.31%) 524.23 (100.15%) 532.35 (101.70%) 526.17 (100.52%)	558.03 (100.29%) 566.90 (101.88%) 574.86 (103.31%) 567.16 (101.93%) 561.19 (100.86%) 558.00 (100.28%) 565.02 (101.55%) 565.38 (101.61%) 557.74 (100.24%) 568.94 (102.25%)	411.39 (101.09%) 410.57 (100.89%) 400.85 (98.50%) 411.19 (101.04%) 407.32 (100.09%) 403.38 (99.13%) 412.08 (101.26%) 420.45 (103.32%) 404.48 (99.40%) 399.49 (98.17%)
n	10	10	10	10
$\bar{X}$	100.4	100.3	101.4	100.3
M	100.4	100.3	101.4	100.3
k	2.4	2.4	2.4	2.4
s	1.01	0.97	1.00	1.55
T	100.0	100.0	100.0	100.0
AV	2.4	2.3	2.4	3.7

The samples complied with the specifications for mass uniformity analysis as well as the weight variation analysis. The outcome of results are comparable and no problems are anticipated when individual tablets are to be used for individual analyses (dissolution).

5.3 Assay of quinine sulfate in quinine sulfate tablets

5.3.1 HPLC as method of analysis - USP monograph

5.3.1.1 Experimental Procedure

The chromatographic conditions, mobile phase, sample and standard solutions specified by the USP monograph for the assay of quinine sulfate tablets were as follows:

Column: 300 x 3.9 mm, C18, 10 µm (µBondapak, Waters)

Mobile phase: A suitably filtered and degassed mixture of water, acetonitrile, methansulfonic acid solution and diethylamine solution in the ratio of 860:100:20:20 (pH adjusted to 2.6 with diethylamine).

Methanesulfonic acid solution: Add 35.0 ml methanesulfonic acid to 20.0 ml of glacial acetic acid and dilute to 500 ml with water.

Diethylamine solution: Dissolve 10.0 ml of diethylamine in water to obtain a 100 ml solution.

Solvent: Mobile phase

Injection volume: 50 µl

Temperature: Ambient (20 - 25ºC)

Flow rate: 1.0 ml/min

Detection wavelength: 235 nm

Reference standard preparation: Transfer about 20 mg of quinine sulfate RS to a 100 ml volumetric flask and dissolve and dilute to volume with mobile phase.

System suitability solution: 0.2 mg/ml of quinine sulfate and 0.2 mg/ml dihydroquinine dissolved in methanol (5% of the final volume) and dilute to volume with mobile phase.

Sample solution: Accurately weigh and powder 20 tablets. Transfer a weighed portion of the powder equivalent to 160 mg of quinine sulfate to a 100 ml volumetric flask. Add 80 ml methanol and shake by mechanical means for 30 minutes. Dilute to volume with methanol. Filter and discard the first 10 ml of filtrate. Transfer 3 ml of the filtrate to a 50 ml volumetric flask and make up to volume with mobile phase.

5.3.1.2 Results

Chromatograms obtained are presented in Figure 5-9 to Figure 5-15. The retention times (± 23 minutes) of quinine sulfate (principal peak) from the sample and standard solution chromatograms corresponded, which implied a positive identification.

Specificity requirements were discussed in section 4.3.6.1 (Chapter 4). The requirements for specificity were met and thus the system was deemed suitable for analysis.

The chromatograms of the mobile phase/solvent (Figure 5-9), sample solution (Figure 5-12 to Figure 5-15), system suitability solution (Figure 5-10) and standard solution (Figure 5-11) showed that there was no interference at the retention times of interest (approximately 23 minutes for quinine sulfate and approximately 33 minutes for dihydroquinine). The aforementioned is a prerequisite since the method use both peaks for the assay calculation (refer to calculation stipulated under the assay procedure, USP quinine sulfate tablet monograph).

Sample solutions were prepared in triplicate, according to the method described in section 5.3.1.1, and each result is the average of the three determinations and the %RSD was calculated for the three determinations.

The assay value was determined using the equation presented in the monograph.

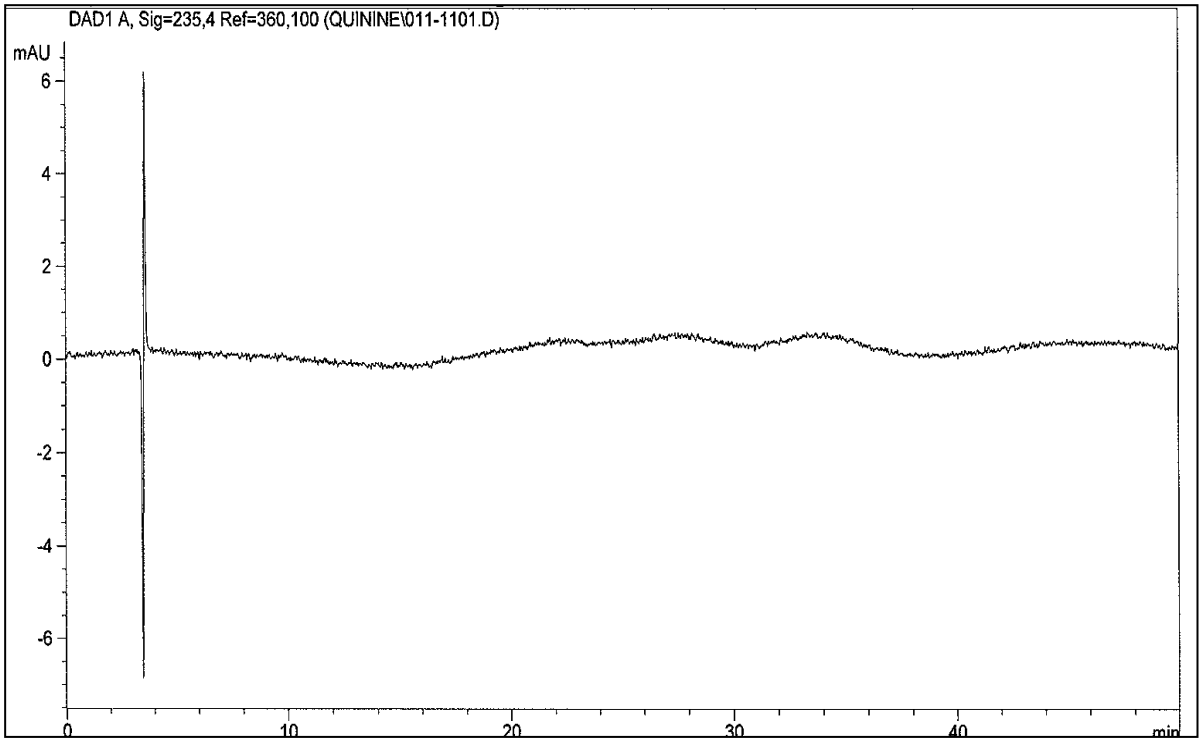

Figure 5-9: Example of a chromatogram of the mobile phase/solvent

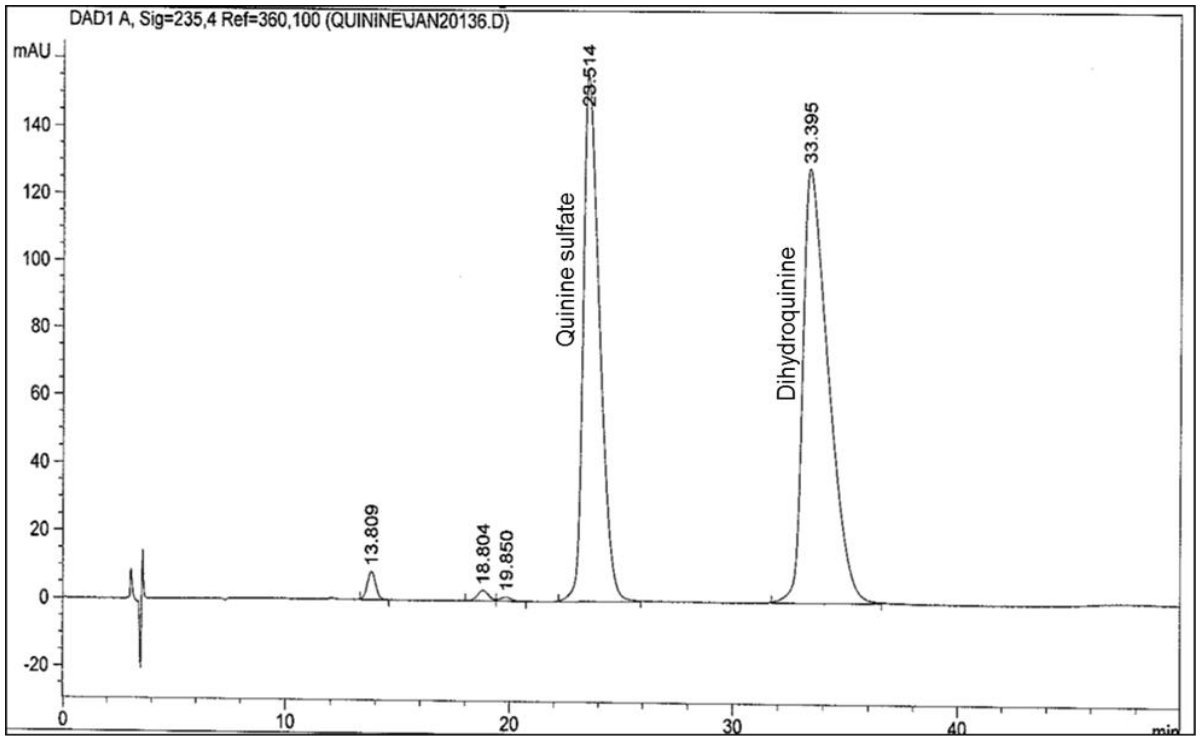

Figure 5-10: Example of a chromatogram of the system suitability solution.

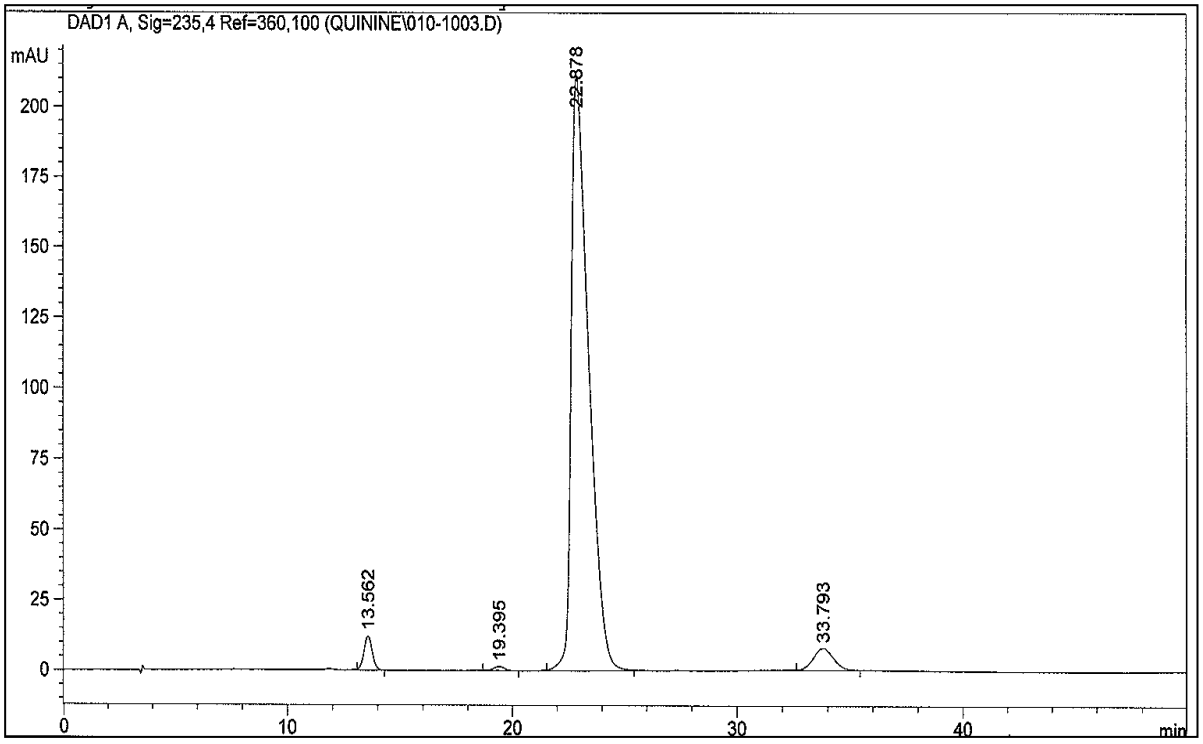

Figure 5-11: Example of a chromatogram of the standard solution.

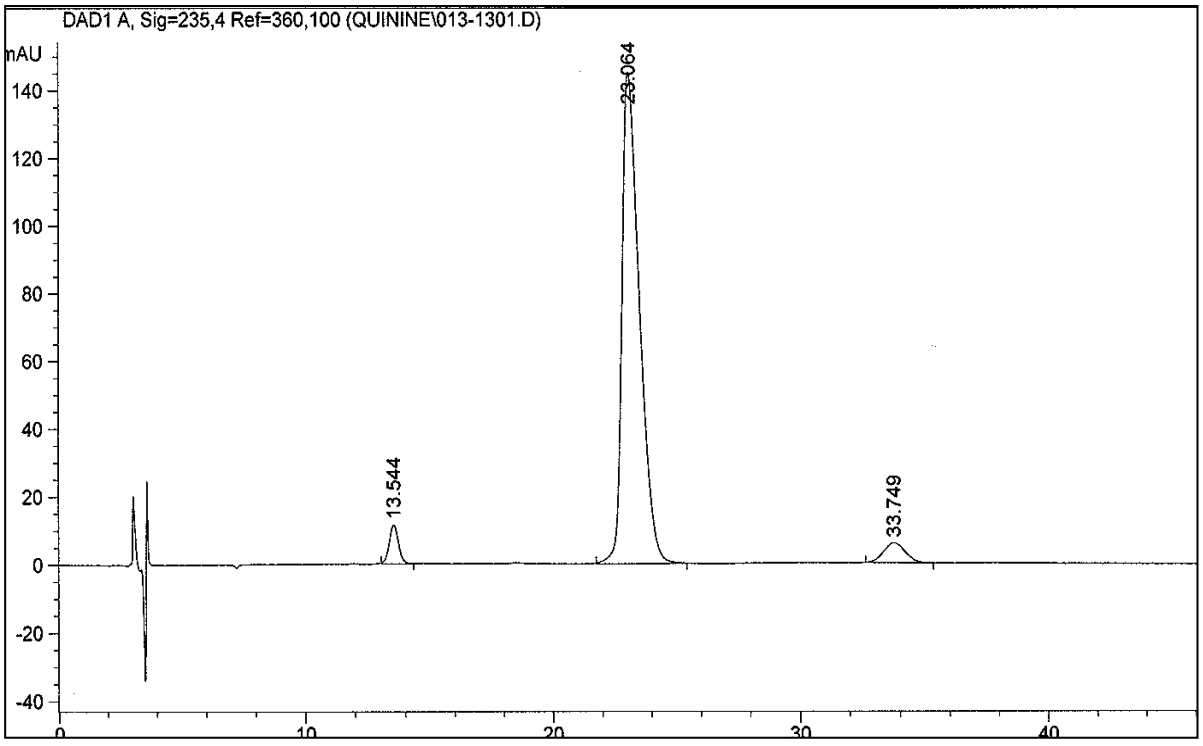

Figure 5-12: Example of a chromatogram of Product 1.

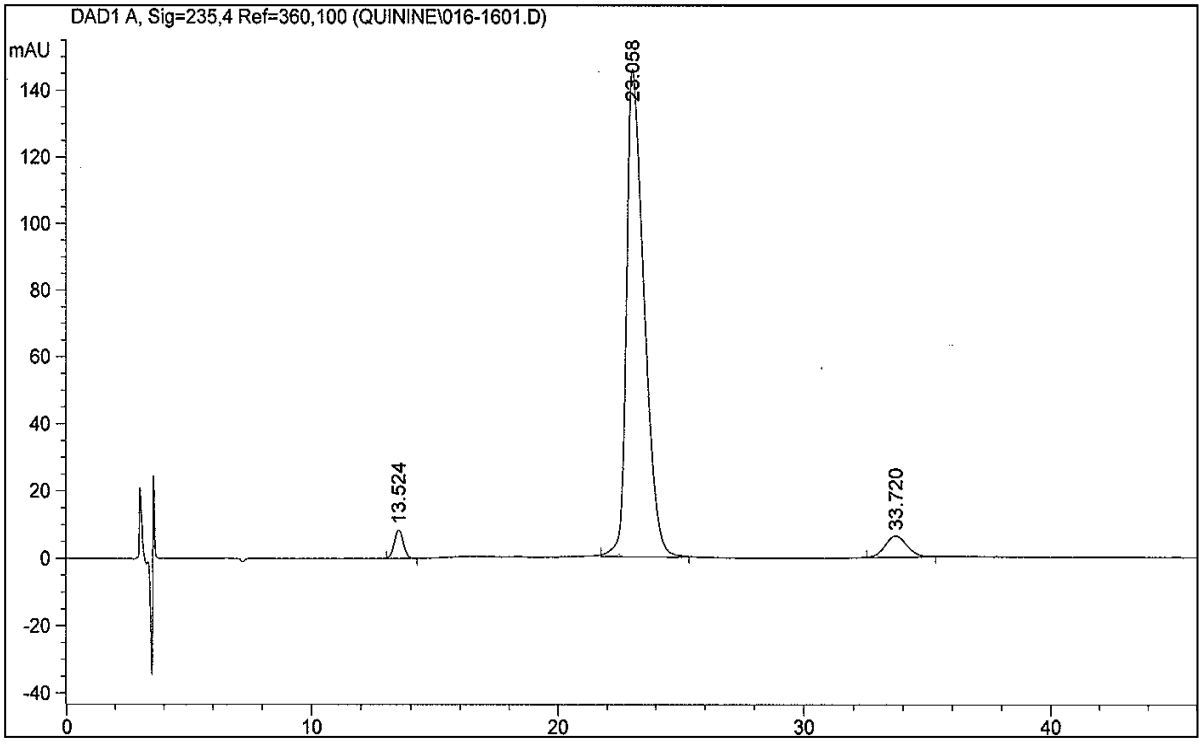

Figure 5-13: Example of a chromatogram of Product 2.

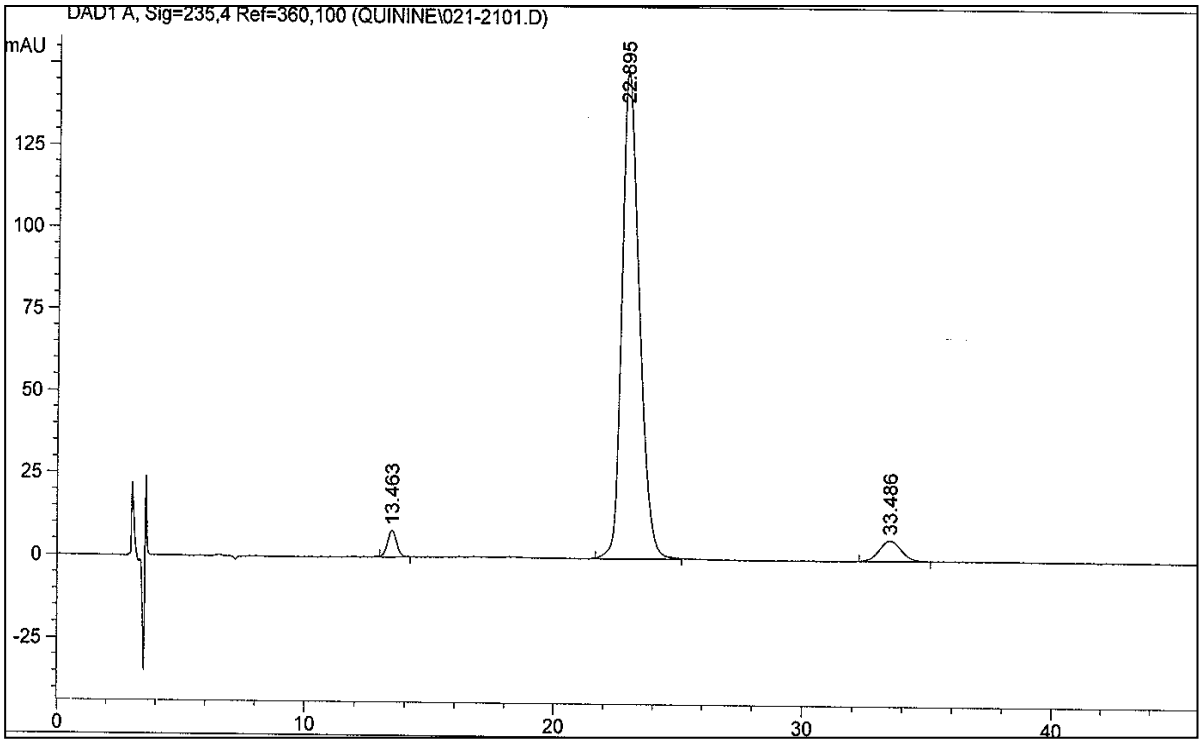

Figure 5-14: Example of a chromatogram of Product 3.

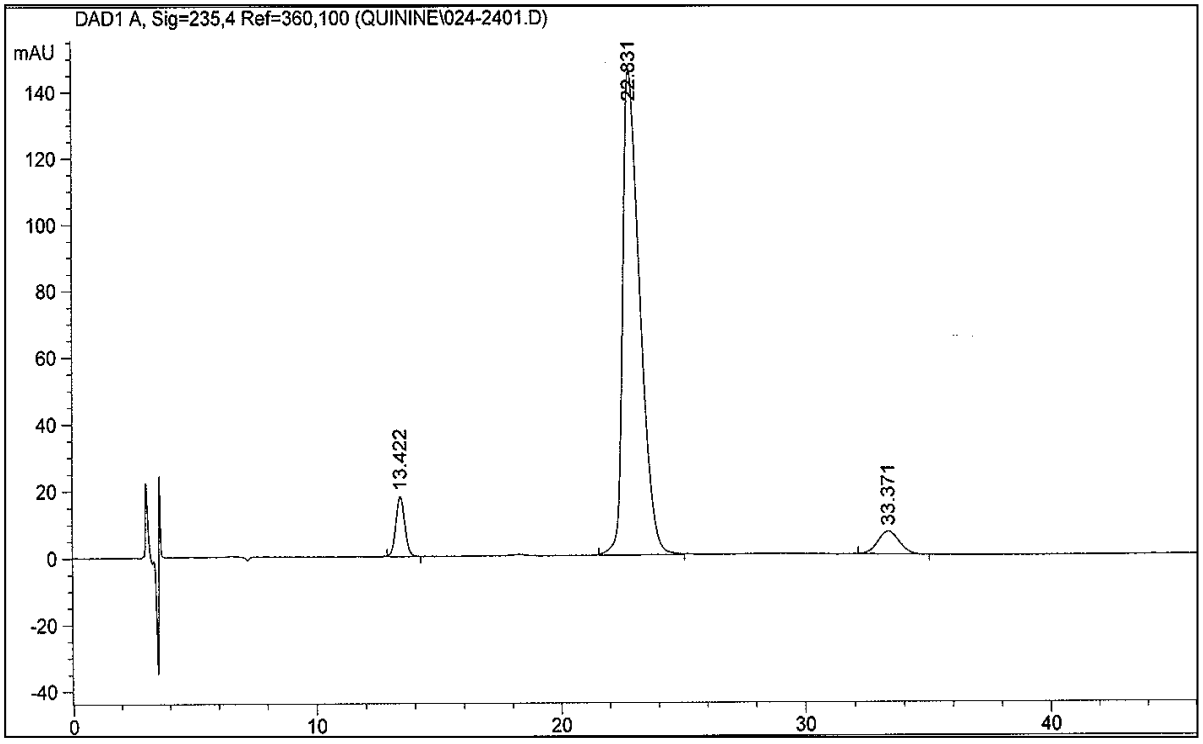

Figure 5-15: Example of a chromatogram of Product 4.

Table 5-13: Assay results for quinine sulfate Products 1 - 4 obtained by HPLC analysis

	Assay		Average Assay % (%RSD)	Compliance statement
	mg quinine sulfate obtained per sample solution	% Assay		
Product 1	159.62 161.66 160.84	99.8 101.0 100.5	100.4 (0.64)	Complies
Product 2	162.39 160.85 158.24	101.5 100.5 98.9	100.3 (1.31)	Complies
Product 3	162.27 163.80 160.76	101.4 102.4 100.5	101.4 (0.95)	Complies
Product 4	160.26 161.13 161.24	100.2 100.7 100.8	100.4 (0.64)	Complies

The assay percentage values for the various products ranged between 98.90% and 102.38%, and complied with the specification of the monograph (refer to Table 5-13). Generally a specification for assay values between 90% and 110% are considered acceptable. The %RSD

values for all the samples were below 2%, indicating that the method produces repeatable results (ICH, 2005).

5.3.2 Non-aqueous titration as method of analysis - BP and *Ph.Int.* method

5.3.2.1 Experimental procedure

Titration is a popular assay technique (Watson, 2005:52). The BP and *Ph.Int.* monograph describe a non-aqueous titration method which is comparable. For reasons explained in Chapter 4, section 4.3.5, the titration method described by the BP monograph was chosen to represent assay by means of titration (the *Ph.Int.* method was not performed). Powdered tablet equivalent to 0.4 g of quinine sulfate was dissolved in 40 ml of acetic anhydride. All reagents used for analysis were prepared as described in the BP monograph. Each ml of the prepared and standardised titrant (0.1 M perchloric acid VS) is equivalent to 29.232 mg of quinine sulfate dihydrate (refer to Chapter 4, section 4.3.5 which discussed the standardisation of the titrant). The assay values were calculated using the standardisation value (1 ml titrant = 29.232 mg quinine sulfate). The results obtained are tabulated in Table 5-14.

$$\%\ quinine\ sulfate = \frac{\text{titre value (ml)} \times 29.232\text{mg} \times 3 \times 100}{4 \times \text{label claim}} \qquad \text{Equation 5.1}$$

5.3.2.2 Results

The percentage assay values for the various products ranged between 98.3% and 102.0%, and complied with the specification of the monograph (refer to Table 5-14). Generally a specification for assay values between 90.0% and 110.0% are considered acceptable. The %RSD values for all the samples were below 2%, indicating that the method produces repeatable results (ICH, 2005).

Table 5-14: Assay results obtained for Products 1 - 4 by non-aqueous titration analysis

	Titre Volume (ml)	Assay		Average % (%RSD)	Specifications (95% - 105%)
		mg quinine sulfate/tablet	% Assay		
Product 1	13.6 13.5 13.6	298.17 295.97 298.17	99.4 98.7 99.4	99.1 (0.43)	Complies
Product 2	13.7 13.7 13.6	300.36 300.36 298.17	100.1 100.1 99.4	99.9 (0.42)	Complies
Product 3	14.0 13.9 13.6	306.94 304.74 298.17	102.3 101.6 99.4	101.1 (1.5)	Complies
Product 4	13.6 13.9 13.8	298.17 304.74 302.55	99.4 101.6 100.9	100.6 (1.11)	Complies

5.3.3 Comparison between the assay results by means of a *t*-test assuming equal variances

An assay analysis was done by means of HPLC analysis and non-aqueous titration analysis as described in section 5.3.1 and 5.3.2 above. The results from each of the four products from HPLC and titration analysis were compared by means of a *t*-test assuming equal variances (Table 5-15). The α-value was taken as 0.05. The *t*-test indicated that there was no statistical significant difference between the HPLC and titration results for Products 2 - 4, however 1.6% difference for Product 1 was deemed statistically different. Based on the results of this study, the greater majority of products did not present a difference between the results obtained (3 out of 4 products) and for this reason the two methods may be deemed similar. It is however acknowledged that the sample pool was limited to only 4 products, and that the possibility still exists that the results may not always be comparable between the two techniques.

Irrespective of the comparability of the actual results, both the techniques provided with the same outcome (all the products complied with the respective specifications of the tests).

The difference between the HPLC assay values and the titration assay values ranged between 0.1 - 1.6 % (Table 5-15).

Table 5-15: Statistical comparison between the assay results from HPLC and non-aqueous titration

	Titration Average (%RSD)	HPLC Average (%RSD)	Difference	*p*-value	Statistical significance
Product 1	98.8 (0.43)	100.4 (0.64)	1.6%	0.02	p<α (not similar)
Product 2	99.5 (0.42)	100.3 (0.31)	0.8%	0.38	p>α (similar)
Product 3	101.8 (0.33)	100.4 (0.95)	1.4%	0.59	p>α (similar)
Product 4	100.3 (1.11)	100.4 (0.64)	0.1%	0.69	p>α (similar)

5.4 Other/Related cinchona alkaloids

5.4.1 HPLC as method of analysis - BP and *Ph.Int.* monograph

5.4.1.1 Experimental procedure

The BP and *Ph.Int.* monographs describe HPLC methods for the quantification of the other/related cinchona alkaloids in quinine sulfate tablets in their respective monographs. The major differences between the two methods were highlighted in Chapter 4, section 4.3.7, (Table 4-16). It was found that the differences between the two methods were within the acceptance limits of allowed variance (robustness). For this reason it was decided to execute the method as specified by the *Ph.Int.* monograph, and the results obtained from this method to be representative of related substances (other cinchona alkaloids) by means of HPLC. The USP monograph test this component (related/other cinchona alkaloids) by means of TLC in conjunction with identification (refer to section 5.1.5.3 for discussion on identification and section 5.4.2 for the discussion on impurities). The outcome of this semi-quantitative test was compared to that of the HPLC *Ph.Int.* method.

The chromatographic conditions, mobile phase, sample and standard solutions specified by the *Ph.Int.* monograph for analysis of the related cinchona alkaloids present in quinine sulfate tablets are as follows:

Column: 150 mm × 4.6 mm, C18, 5 µm (Luna, Phenomenex)

Mobile phase: 6.8 g potassium dihydrogen orthophosphate and 3.0 g of hexylamine in 900 ml water, adjusting the pH to 3.0 with orthophosphoric acid and dilute to 1000 ml with water. Mix 920 ml of this solution with 80 ml of acetonitrile.

Flow rate: 1.3 ml/min

Injection volume: 10 µl

Detection wavelength: 316 nm

Solvent: repared using 80 volumes of water, 20 volumes acetonitrile and 0.1 volume of orthophosphoric acid.

Solution 1: Transfer tablet powder containing equivalent 60 mg of quinine sulfate into a 20 ml volumetric flask. Add 15 ml of solvent and sonicate for 5 minutes. Allow to cool to room temperature and dilute to volume with solvent. Filter using a 0.45 µm Millipore filter.

Solution 2: Transfer 30 mg of quinine sulfate reference standard to a 10 ml volumetric flask. Dilute to volume. Filter using a 0.45 µm Millipore filter.

Solution 3: Transfer 15 mg of quinidine sulfate reference standard to a 5 ml volumetric flask. Dilute to volume using solution 2 as solvent. Filter using a 0.45 µm Millipore filter.

5.4.1.2 Results

The monograph specifies the following relative retention times with reference to quinine; cinchonidine about 0.6, quinidine about 0.8, dihydroquinidine about 1.2 and dihydroquinine about 1.5. All the peaks that eluted during the analysis were identified (Figure 5-18 = solution 3 = resolution solution) and were found to correspond with that specified by the monograph (also see Chapter 4, section 4.3.7.1.3).

The percentage content of the related substances in the sample solution was calculated by normalisation. The content of dihydroquinine should not be more than 10% and the content of cinchonidine not more than 5%. If any other related substance was detected the content thereof should not be more than 2.5%. The related substances in total should not be more than 15%. Any related substance of content less than 0.1% was disregarded.

Table 5-16 summarises the results obtained for the dihydroquinine and cinchonidine content (Figrue 5-20, Figure 5-21, Figure 5-22 and Figure 5-23). The sum of all the related substances in all four products was below 15% - refer to Table 5-16. No other related substances except for dihydroquinine and cinchonidine were detected.

All products were found to be within specifications except for Product 4, which had a cinchonidine content of more than 5% as seen in Figure 5-23/Table 5-16.

Table 5-16: The results obtained when testing for related cinchona alkaloids present in quinine sulfate tablets - *Ph.Int.* monograph

	Cinchonidine Average (%RSD)	Quinidine sulfate	Dyhydro-quinidine	Dihydroquinine Average (%RSD)	Total %
Product 1	4.5 (0.07)	NPD	NPD	4.7 (0.11)	9.2
Product 2	3.3 (0.47)			5.0 (0.25)	8.3
Product 3	3.1 (0.44)			5.3 (10.88)	8.4
Product 4	6.8 (0.18)			5.3 (0.11)	12.1

* NPD = no peaks detected

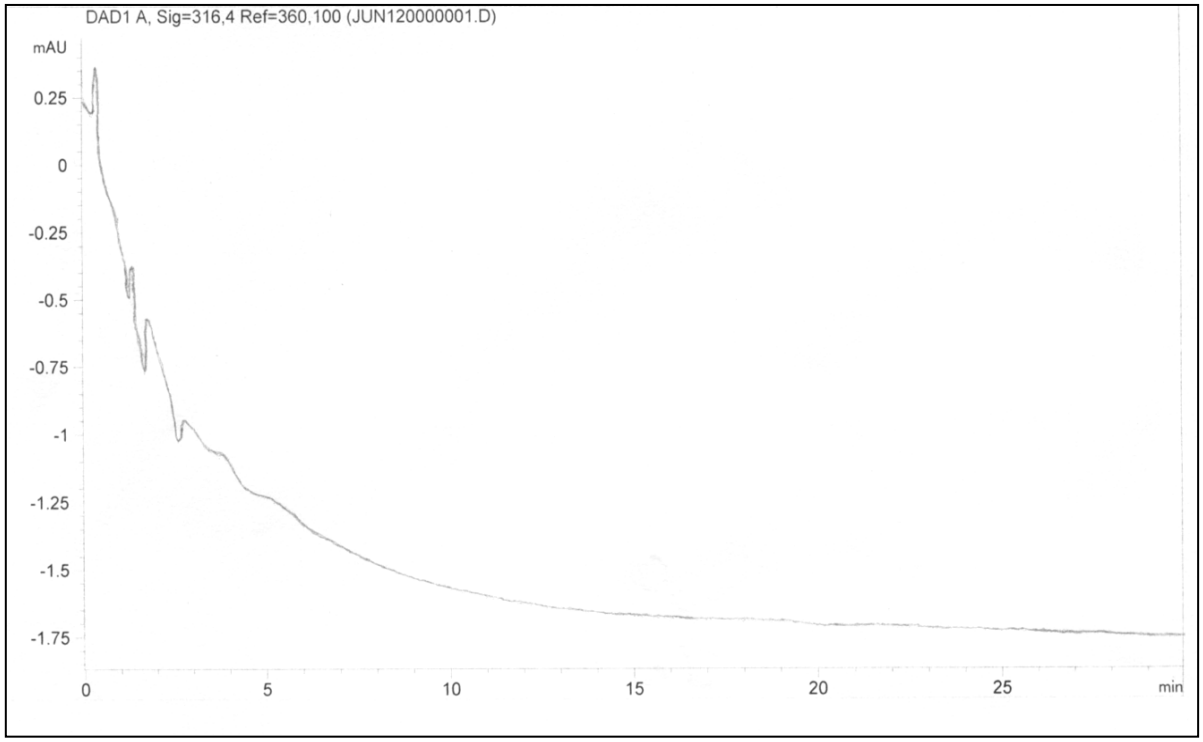

Figure 5-16: Example of a chromatogram of the mobile phase.

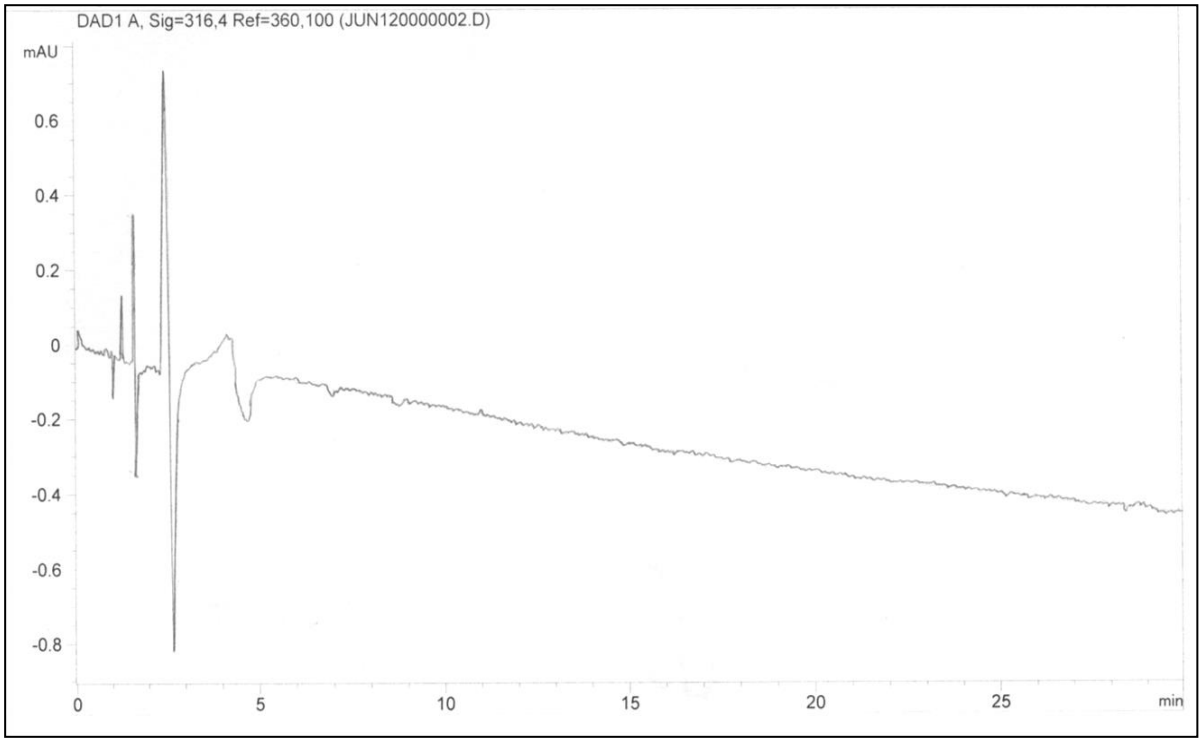

Figure 5-17: Example of a chromatogram of the solvent.

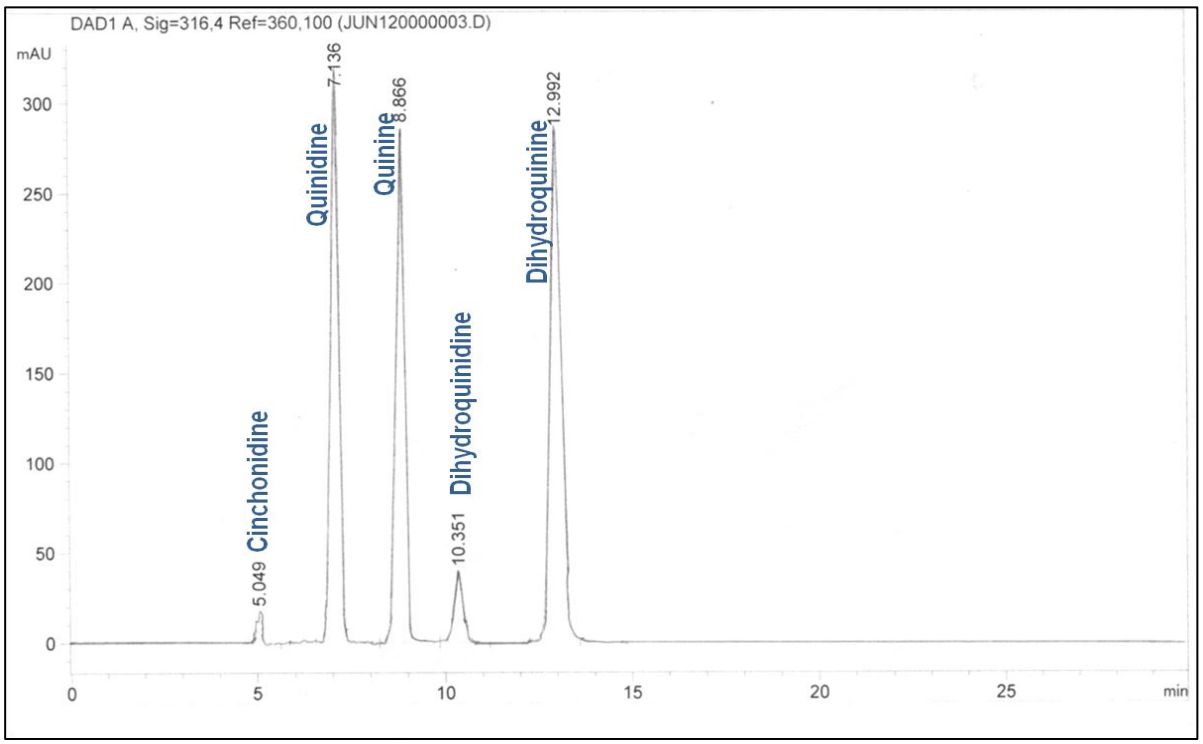

Figure 5-18: Example of a chromatogram of the resolution solution (solution 3).

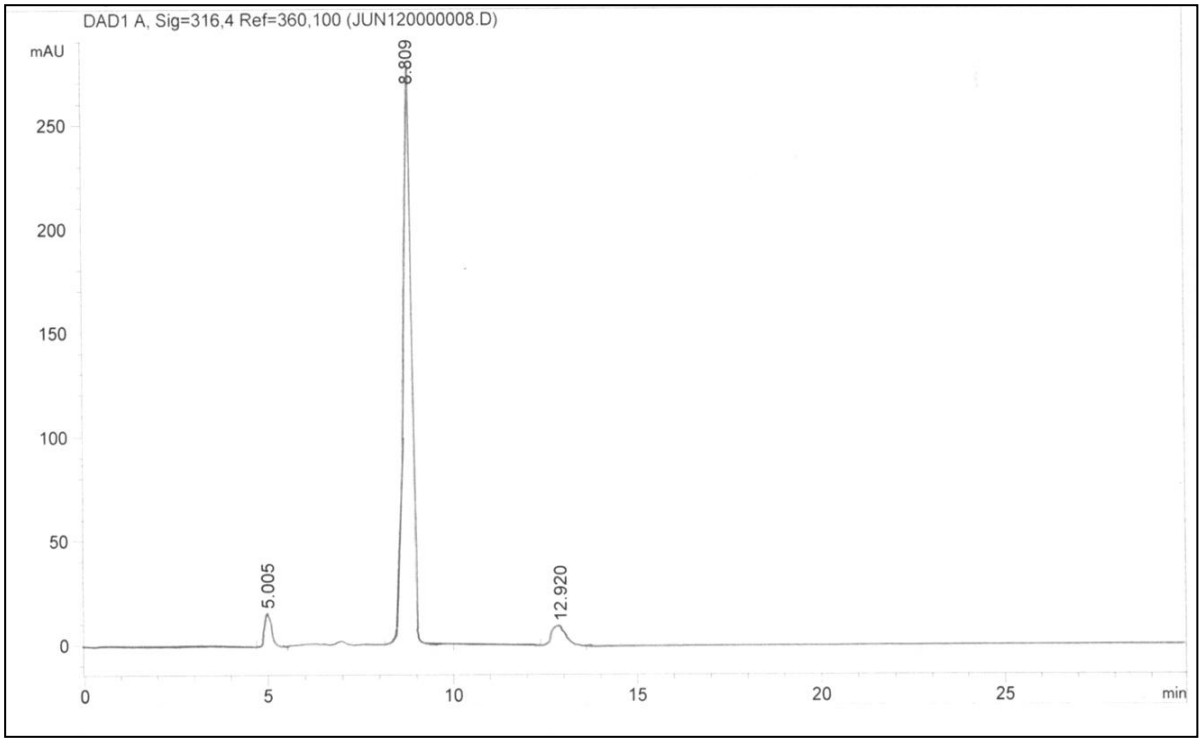

Figure 5-19: Example of a chromatogram of the 100% standard solution (solution 2).

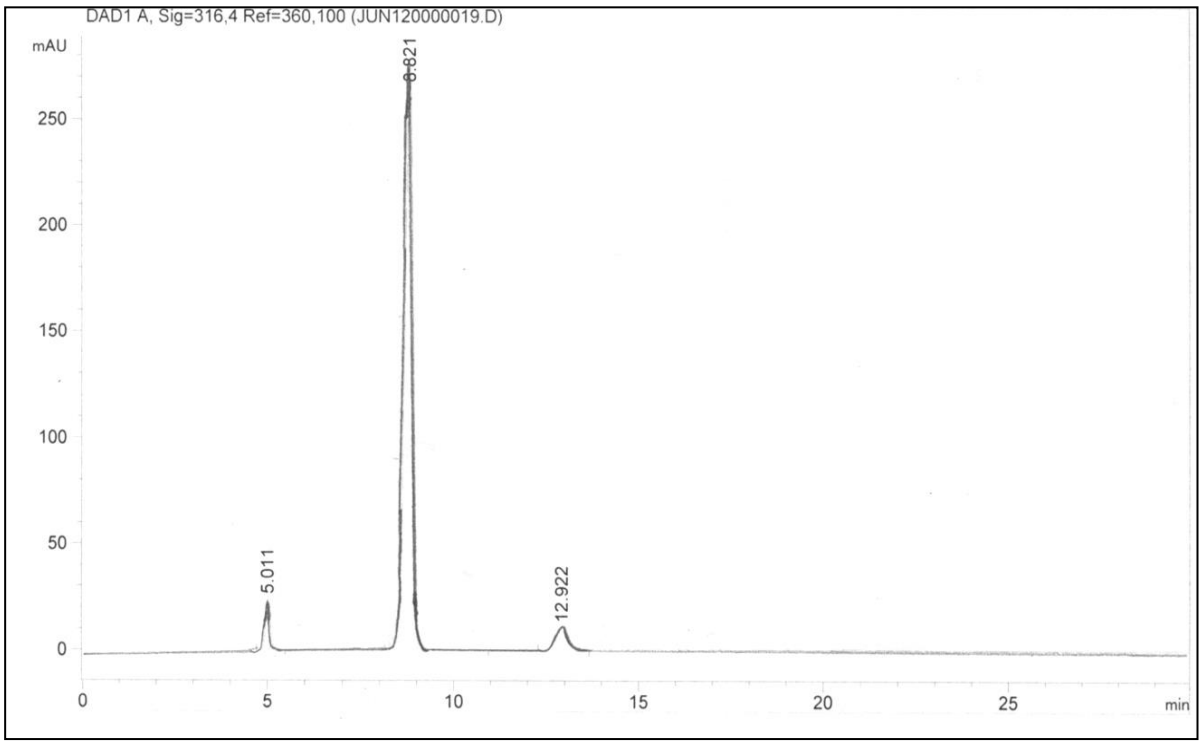

Figure 5-20: Example of a chromatogram of Product 1 (solution 1).

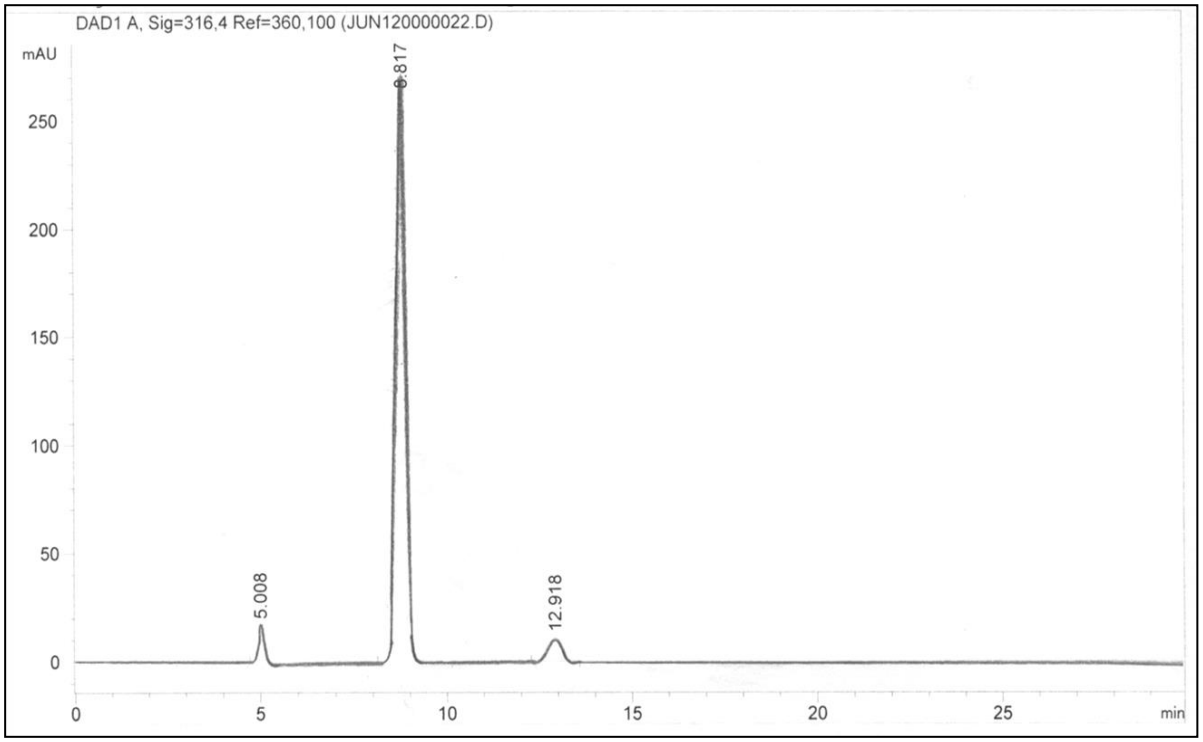

Figure 5-21: Example of a chromatogram of Product 2 (solution 1).

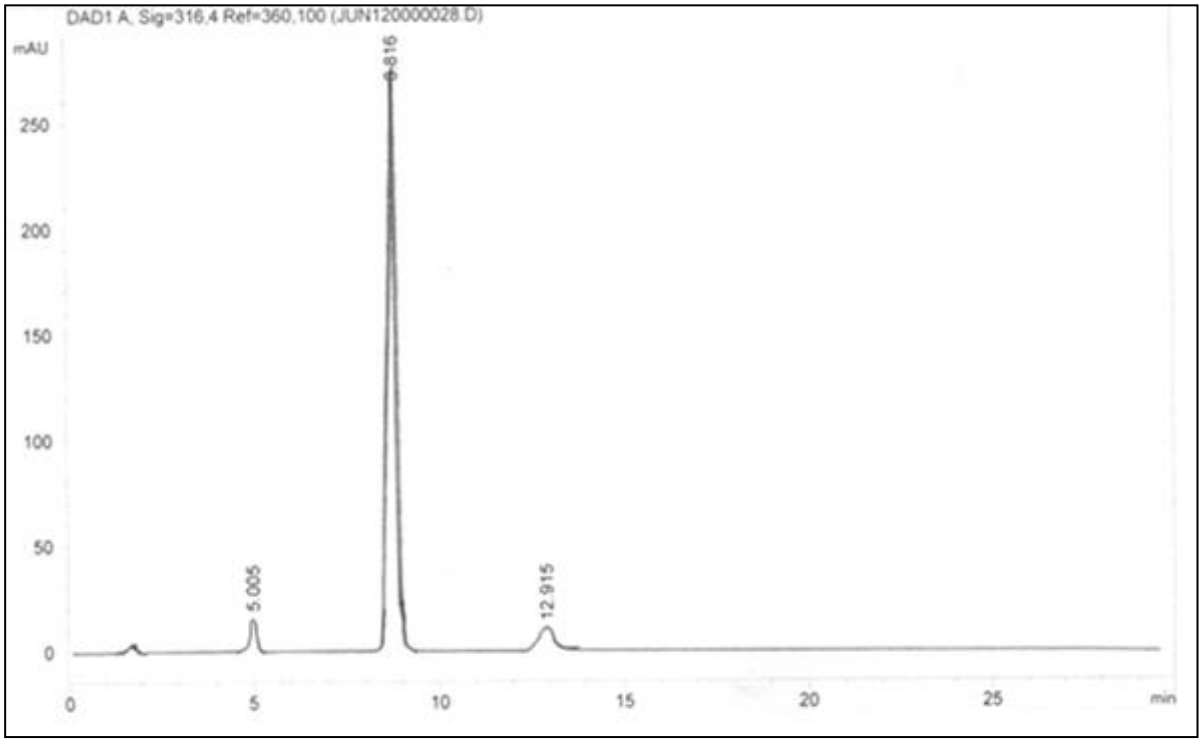

Figure 5-22: Example of a chromatogram of Product 3 (solution 1).

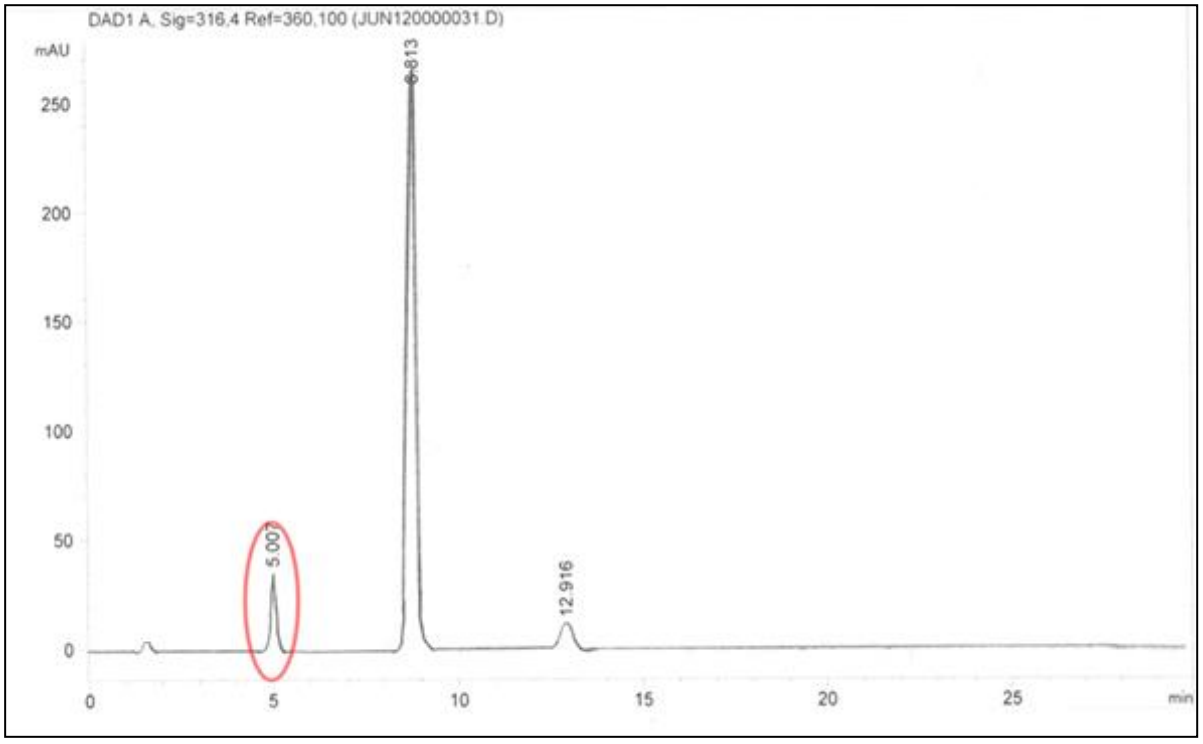

Figure 5-23: Example of a chromatogram of Product 4 (solution 1).

5.4.2 TLC analysis

The USP monograph employs a TLC method for simultaneous identification of quinine as well as testing for related cinchona alkaloids. The chromatographic conditions and results of the USP monograph TLC method have been stated in section 5.1.5.3. In addition to that described in 5.1.5.3, the USP monograph for quinine sulfate tablets have the following specifications for the related cinchona alkaloids test. The TLC plate is presented in Figure 5-24.

After being sprayed with glacial acetic acid any spot produced by the sample solution at the R_f value (spot position) of a spot produced by the Standard solution B is not greater in size or intensity than that corresponding spot. Apart from these spots and from the spot appearing at the R_f value of quinine, any additional fluorescent spot is not greater in size or intensity than the spot of Standard solution A. After sprayed with potassium iodoplatinate any spot produced by the sample solution is not greater in size and intensity than a corresponding spot from Standard solution B.

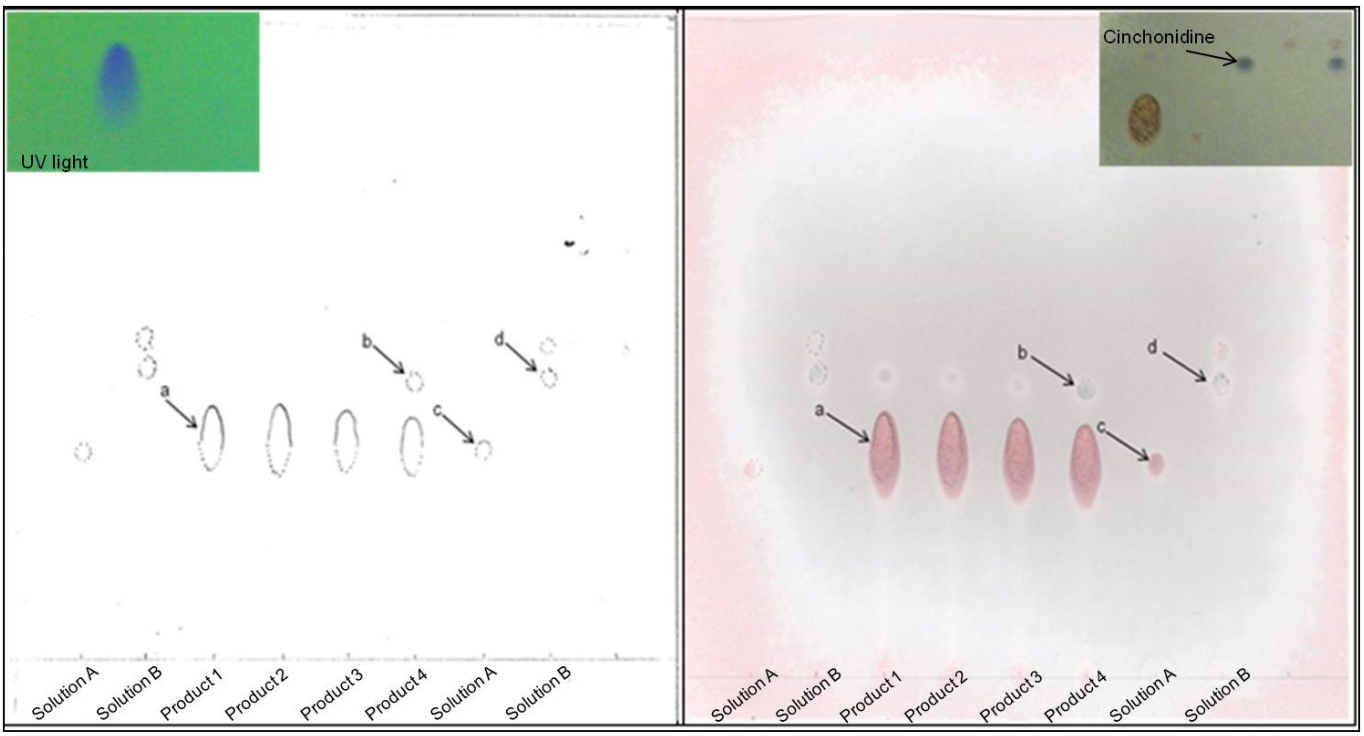

Figure 5-24: **The TLC plate after sprayed with glacial acetic acid (left). The TLC plate after sprayed with potassium iodoplatinate spray reagent (right) where (a) represents the spot for quinine present in the samples, (b) represents the spot for cinchonidine present in the samples, (c) represents the spot of the quinine present in standard solution A and (d) represents the spot for cinchonidine in standard solution B. The individual spots were identified as seen in the insert on the right where cinchonidine is represented by the purple.**

The API's were spotted separately and identified as seen in Figure 5-24 (insert on the right). This was done to ensure the correct identification of constituents of Solution B containing both cinchonidine and quinidine. An example of how the quinine spot fluoresces when viewed under the 254 nm UV light is shown in Figure 5-24 (insert on the left).

The quinine sulfate spots from the standard solution were less intense than that of the sample solutions, since the quinine sulfate concentration (0.06 mg/ml quinine sulfate) is less than the sample solution (6 mg/ml quinine sulfate) - refer to sample and standard preparations in section 5.1.5.3. All four products presented with cinchonidine spots. The cinchonidine spots from Product 1, 2 and 3 were less intense than that of standard solution B and complied with the first requirement. The cinchonidine spot of the sample solution of Product 4 was more intense than that of standard solution B and for this reason did not comply with the first requirement for the

test (Figure 5-24). This finding (out of specification for Product 4) correlated with the out of specification results obtained using the HPLC method of the *Ph.Int.* No other spots were produced by the sample solutions, indicating that the rest of the test requirements were met for all the products.

5.4.2.1 Correlation between the HPLC (*Ph.Int.* monograph*)* and TLC (USP monograph) results for related substances/other cinchona alkaloids

The TLC method is a semi-quantitative method, whereas the HPLC method is a quantitative method, but the outcome of the tests may still be compared. The related substances results from the *Ph.Int.* monograph HPLC method and the USP monograph TLC method are summarised in Table 5-17.

Table 5-17: Related substances results from the *Ph.Int.* monograph HPLC method and the USP monograph TLC method

Product	USP (TLC)	*Ph.Int.* (HPLC)	Outcome
1, 2, 3	Chinchonidine detected < specification. No other related substance (spot) detected.	Chinchonidine detected, but at a concentration lower than the upper limit of the monograph (3.55% < 5%). Dihydroquinine detected < specification. No other related substance detected.	Comply
4	Cinchonidine detected > specification No other related substance (spot) detected.	Chinchonidine detected at a concentration more than the upper limit of the monograph (5.0% < 6.8%). Dihydroquinine detected < specification. No other related substance detected.	Doesn't comply

From Table 5-17 it can be seen that Products 1 - 3 complied with the specifications for both tests (TLC and HPLC), whereas Product 4 did not comply with the specifications of either test for cinchonidine. Despite the fact that the other parameters of Product 4 is within specification, it does not affect the final outcome of the test (still out of specification, all parameters must be within specification). TLC may not be as sensitive as the HPLC method (the TLC method detected only cinchonidine, whereas the HPLC detected cinchonidine and dihydroquinine), but rendered comparable outcomes (i.e. Product 4 faliling to comply with set specifications) in

comparison with HPLC. The superior sensitivity of the HPLC over TLC resulted in the detection of dihydroquinine. The levels of the detected dihydroquinine were however less than the allowed limits thereof. For this reason one can contemplate that should the dihydroquinine have presented in the samples in concentrations exceeding the allowed limits, it would have resulted in spots on the TLC plate that might have been identified and found more intense than the standard solution.

Although HPLC and TLC are two different types of chromatography, both tests were specific enough to detect the presence of related cinchona alkaloids. The same outcome (i.e. Products 1 – 3 comply, Porduct 4 does not comply) was obtained and rendered these two tests equally fit for testing for related cinchona alkaloids.

5.5 Identification by means of HPLC analysis

The USP monograph (from the assay procedure) and *Ph.Int.* monograph (from the related substances test) rely on the identification of quinine sulfate by means of retention time comparison between the sample and standard solutions. The chromatograms obtained from the assay testing (USP monograph) shown in Figure 5-10 (system suitability), Figure 5-11 (standard solution), Figure 5-12 (Product 1), Figure 5-13 (Product 2), Figure 5-14 (Product 3) and Figure 5-15 (Product 4) showed that the principal peaks (quinine sulfate) in the sample solutions corresponded with that of quinine sulfate in the standard solutions (retention time of ± 23 minutes), and thus rendered a positive identification for quinine sulfate.

The chromatograms obtained from the related cinchona alkaloid analysis (*Ph.Int*.) shown in Figure 5-18 (resolution solution), Figure 5-19 (standard solution), Figure 5-20 (Product 1), Figure 5-21 (Product 2), Figure 5-22 (Product 3) and Figure 5-23 (Product 4) also showed a positive identification for quinine sulfate. The retention time of the principal peak (quinine sulfate) seen in the chromatogram of the standard solution compared to the principal peak seen in the chromatograms of the samples at ± 8.8 minutes.

Both HPLC methods/techniques were found equal in their ability to identify quinine sulfate.

Conclusion

The BP, USP and *Ph.Int.* each present with a quinine sulfate tablet monograph. The different monographs employ different combinations of techniques and methods (Table 5-1.)

Although there were similarities identified between the means of identification of quinine sulfate (methods and techniques) from the different monographs, they do not all employ the same

combinsation of tests. As mentioned in section 5.1, a combination of identification tests is required to address the specificity of identification tests.

From Table 5-1 it can be seen that all three monographs employ TLC as an identification test. All products were tested using each TLC identification test. Despite the differences in these methods, the results produced by each of the methods confirmed that the method was suitable for its purpose. All the TLC identification tests showed positive identification of quinine sulfate in all the products, and adequate specificity between similar compounds was achieved (as required for each respective test).

The USP and *Ph.Int.* monographs are the only monographs that list HPLC methods for identification. Both rely on the retention time of quinine sulfate in the sample solution vs quinine sulfate in the reference standard solution. All products tested positive by means of HPLC. This showed that despite the differences in the HPLC methods, the same outcome was achieved.

The *Ph.Int.* monograph is the only monograph presenting an UV method for identification. All products were compliant with the requirements thereof.

The USP monograph is the only monograph presenting with a fluorescence test for identification of quinine sulfate. All products were compliant with the requirements thereof.

The BP and *Ph.Int.* monographs provide identification of quinine sulfate by means of pH-test. The main difference between the methods were in the quality of water (purified vs. carbon dioxide free water) that is specified for use. Although the pH-test results differed between the two methods, both sets of results were compliant with what was expected by the different monographs. The difference in the allowed specifications (and the results that accompanied the difference) was justified by the fact that the pH of the different grades of water influences the limits and results accordingly.

All monographs specify the identification of sulfate by means of a sulfate identification test. These different methods are comparable in principal and it was found that all the products complied with the respective requirements thereof. Although the sulfate test methods differed slightly, they were deemed specific enough for the identification of sulfate in quinine sulfate.

The BP and *Ph.Int.* monograph both employed non-aqueous titration methods that only differed in the detection of the end-point (potentiometrically vs. indicator). For reasons explained in Chapter 4 (section 4.3.5) the non-aqueous titration tests of the BP method were executed and the results thereof representative of assay by means of non-aqueous titration. The assay results obtained using the titration method were compared to that obtained using the HPLC

quantitative method of the USP monograph. The *t*-test assuming equal variance showed that there was no statistical difference between the results of Products 2, 3 and 4, however the results of Product 1 were found to be statistically different between the HPLC and titration methods. The outcome of the tests were however comparable, since all products complied with the specifications.

From the results it was concluded that although the methods and techniques are different between the monographs, the outcomes (i.e. compliance or non-compliance to the specifications) were found to be the same. In Chapter 6 the release of the quinine sulfate API from the solid oral dosage form (tablets) will be investigated.