Chapter 7: Summary and conclusion

A schematic outline of this study is presented in Figure 7-1.

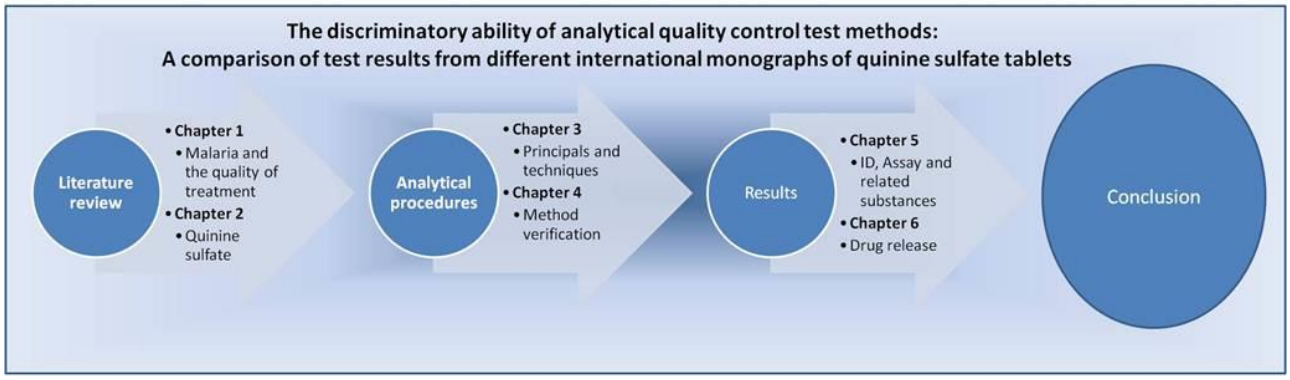

Figure 7-1: Schematic outline of this study

From the literature overview (Chapters 1 and 2) it is evident that malaria is responsible for a high death toll, despite a wide variety of known pharmacological treatments available. The failure of treatment is seemingly the highest in developing countries, and these countries also suffer the highest occurrence of counterfeit and substandard medicines (due to a lack of QC measures). The use of substandard medicine may not only result in ineffective treatment (and death), but may also result in growing resistance against treatment.

Quinine sulfate was the anti-malarial evaluated in this study, since some surveillance studies have shown it to be at risk of being substandard. Quinine sulfate remains a popular anti-malarial treatment as it is still regarded as being highly effective, fairly inexpensive and has little to no reported resistance. By ensuring the quality of quinine sulfate tablets, one can minimise the risk of this treatment becoming obsolete in malaria treatment, by minimising the potential of resistance to develop.

Various means are employed to ensure the quality of medicines, such as analytical QC procedures (referred to as monographs). These monographs should provide stringent, yet justified tests and specifications to evaluate the quality of medicines.

This study set out to critically evaluate the three quinine sulfate tablet monographs to ensure that they be true in their respective ability to discriminate between products which are of acceptable quality and those of inferior quality. These monographs employ different analytical techniques and combinations of methods to evaluate the quality of quinine sulfate tablets

(Table 7-1). Amongst the highlights of this study was to identify and address any potential indiscretion between the monographs.

To ensure that accurate and trustworthy results are obtained, it was necessary to first gain knowledge on the theories and applications of all the techniques and apparatus which are utilised by the different quinine sulfate tablets monographs (Chapter 3) – Table 7-1. The review of all the required instrumentation and techniques thereof ensured that all experimental work were performed in keeping with GLP procedures and in adherence to the respective procedures of the monographs.

Table 7-1: Summary of analytical QC tests for the quinine sulfate tablets monographs of the USP, BP and *Ph.Int.*

Test	Technique	USP	BP	Ph.Int.
ID	TLC	✓	✓	✓
	HPLC	✓	✗	✓
	UV	✗	✗	✓
	Fluorescence	✓	✗	✗
	pH	✗	✓	✓
	Sulfate precipitation	✓	✓	✓
Assay	HPLC	✓	✗	✗
	Non-aqueous titration	✗	✓	✓
Related substances (other cinchona alkaloids or chromatographic purity)	HPLC	✗	✓	✓
	TLC	✓	✗	✗
Uniformity of dosage units	UV/Vis	✓	✗	✗
Dissolution	UV/Vis	✓	✓	✓
Disintegration	Disintegration	✗	✗	✓

Even though monographs found in pharmacopoeias are considered to be validated, it is still necessary to verify that they perform as intended under the specific conditions of the laboratory in which the tests are to be performed (method verification). The quantitative methods that were verified for the quinine sulfate tablet monographs were:

- dissolution tests
- assay tests
- related cinchona alkaloids/other cinchona alkaloids (related substances)

The results presented in Chapter 4 found all methods to be compliant with the method requirements (Table 7-2), and therefore considered to be successfully transferred and suitable

for subsequent use. Those parameters not evaluated (Table 7-2) are not considered for the purposes of method verification, but for complete method validation.

Table 7-2: Summary of the method verification parameters that were evaluated and discussed in Chapter 4

	Method validation parameters							Method verified and found suitable for use	
	Specificity	Precision and repeatability	Accuracy and recovery	Linearity and range	Robustness	Detection limit	Quantitation limit	Yes	No
Dissolution									
from USP monograph	●	●	●	●				●	
from BP monograph	●	●	●	●				●	
from *Ph.Int.* monograph	●	●	●	●				●	
from Acetate buffer studies	●	●	●	●				●	
Assay									
from USP monograph (HPLC)	●	●	●	●				●	
from BP monograph (non-aqueous titration)		●	●	●				●	
Related cinchona alkaloids									
from *Ph.Int*. monograph	●	●	●	●		●			

Chapter 5 set out to discuss the results of ID, assay and related substances testing. It was evident that even though the monographs employ different combinations and/or means for identification, assay and related substance testing, they were either found equal or justified for their differences without impairing their unique ability to present accurate outcomes. The outcomes between the monographs were also deemed similar (Table 7-3).

Table 7-3: Summary of ID, assay and related substance test results

Test	Technique	Specification	Product	USP	BP	*Ph.Int.*
Identification	Various (see Table 7-1)	Positive for quinine sulfate as per respective monograph ID test	1 2 3 4	Complies	Complies	Complies
Assay	HPLC	90.0 – 110.0 %	1 2 3 4	100.4% (0.64%) 100.3% (1.31%) 101.4% (0.95%) 100.4% (0.64%)	N/A	N/A
	Non-aqueous Titration	95.0 – 105.0 %	1 2 3 4	N/A	99.1% (0.43%) 99.9% (0.42%) 101.1% (1.5%) 100.6% (1.11%)	*
Related substances (other cinchona alkaloids or chromatographic purity)	HPLC	Refer to Chapter 5 (section 5.4)	1	N/A	**	C = 4.5%, DHQ = 4.7% Total = 9.2%
			2			C = 3.3%, DHQ = 5.0% Total = 8.3%
			3			C = 3.1%, DHQ = 5.3% Total = 8.4%
			4			C = 6.8%, DHQ = 5.3% Total = 12.1%
	TLC	*Rf value (spot position) of a spot produced by the Standard solution is not greater in size or intensity than that corresponding spot in the sample solution. Apart from these spots and from the spot appearing at the Rf value of quinine, any additional fluorescent spot is not greater in size or intensity*	1 2 3 4	Complies Complies Complies Does not comply	N/A	N/A

C = Cinchonidine, DHQ = Dihydroquinine * not performed. considered similar to BP assay method ** not performed. considered similar to *Ph.Int.* related substances method

All the monographs employ TLC as an identification test. Despite the differences in these methods, the results found each method to be suitable for its purpose and all samples tested positive for quinine sulfate.

The BP and *Ph.Int.* provide with similar means to positively identify quinine sulfate by means of pH. The main difference between the methods were in the quality of water (normal purified water vs. carbon dioxide free water) that is specified for use. Although the pH results differed between the two methods, both sets of results were compliant with the different specifications of the different monographs. The difference in the allowed specifications (and the results that accompanying the difference) was justified by the fact that the pH of the different grades of water influence the limits and results accordingly.

Since quinine may present in forms other than quinine sulfate (such as quinine dihydrochloride) it is understandable to identify the specific form in which it presents (in this case sulfate). All monographs specified the identification of sulfate by means of a sulfate identification test. The methods presented in the different monographs are comparable in principle and all products found compliant 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 (in conjunction with the range of other identification tests performed).

The other identification tests, specific to the monograph (UV – *Ph.Int*., Fluorescence – USP) found all products compliant with the respective specifications and increased the level of specificity regarding the identification of quinine sulfate in these monographs.

The assay results from non-aqueous titration (*Ph.Int*.) were compared with the results from the HPLC assay test method described by the USP. The *t*-test assuming equal variances showed that there was limited statistical difference between the result sets of the titration vs. HPLC methods. From the results it was derived that although the assay methods and techniques are different between the monographs, the outcomes were found to be comparable

The results from the related substances HPLC test procedure of the *Ph.Int.* were compared with the results from the TLC method of the USP. The results unified in the same final outcomes. Although the HPLC is a quantitative (exact values can be obtained) method and the TLC method only semi-quantitative (intensity of spots are related to concentrations) both techniques found Products 1 - 3 to comply with the required limits thereof, whereas the cinchonine result of Product 4 did not meet the criteria thereof by both methods/techniques.

Chapter 6 discussed the results from API release testing (dissolution and disintegration). Dissolution testing of Products 1 - 4 indicated that the outcomes of these methods differed. All

the products complied to the respective specifications of the BP and USP, but not with that of the *Ph.Int*. The *Ph.Int.* specifies dissolution as well as disintegration testing for analysis of quinine sulfate release testing from the solid oral dosage form. The outcome of the disintegration testing was compliant which was in contrast of the dissolution testing which was non-compliant. Possible reasons as to why these outcomes differed were investigated.

According to Lindenberg *et al.* (2004:267) quinine sulfate may either be classified as BCS class I or III by implying that quinine sulfate is highly soluble in physiological media ranging from pH 1.2 – pH 6.8. This did not correspond with the dissolution results. From dissolution results it was anticipated that the solubility of quinine sulfate be indirectly proportional to pH. As part of the investigation, solubility studies were performed. It was found that quinine sulfate is highly soluble in 0.1 M hydrochloric acid (pH 1.2) but not in phosphate buffer (pH 6.8). Based on the solubility results from this study, quinine sulfate may not be regarded as highly soluble in the complete physiological pH range. This outcome gave reason to believe that incorrect or inconclusive results regarding the solubility of quinine sulfate was used as starting point for the development of the *Ph.Int.* dissolution method. The study was furthered by performing dissolutions using alternative dissolution parameters (different medium, medium volume etc.) in an attempt to present a more suitable dissolution method. Different dissolution methods (developmental studies) were investigated (Table 7-4).

Table 7-4: The different developmental phase dissolution parameters that were proposed for the study

Dissolution conditions	Dissolution medium	Dissolution volume	Agitation (rpm)	Apparatus
Developmental study 1	Acetate buffer,pH 4.5	900 ml	75 rpm	Paddle
Developmental study 2	Acetate buffer,pH 4.5	500 ml	75 rpm	Paddle
Developmental study 3	Acetate buffer,pH 4.5	500ml	50 rpm	Paddle
Developmental study 4	Phosphate buffer, pH 6.8	900 ml	75 rpm	Paddle
Developmental study 5	Phosphate buffer, pH 6.8	1000 ml	75 rpm	Paddle
Developmental study 6	Phosphate buffer, pH 6.8	900 ml	100 rpm	Paddle

A summary of outcomes of the developmental studies are shown in Table 7-5 and Figure 7-2.

Table 7-5: Summary of the outcomes of the Developmental studies

Developmental study	Outcome
1	Lacked discriminatory ability. All products achieved ±95% dissolution within 15 minutes. Similar to dissolution performance found with USP and BP methods.
2	Products 3 and 4 achieved > 85% within 15 minutes. The discriminatory ability was not considered completely satisfactory.
3	Products presented with coning which resulted in incomplete dissolution and large variance.
4	All products achieved improved dissolution, but Product 4 showed a high variance (%RSD) and none of the profiles showed a plateau phase, indicating dissolution was still incomplete after 60 minutes.
5	The f_2-values indicated that there was no statistical improvement in the dissolution performance in comparison with developmental study 5 (100 ml extra medium).
6	Allowed the best dissolution conditions for the samples while maintaining discriminatory ability.

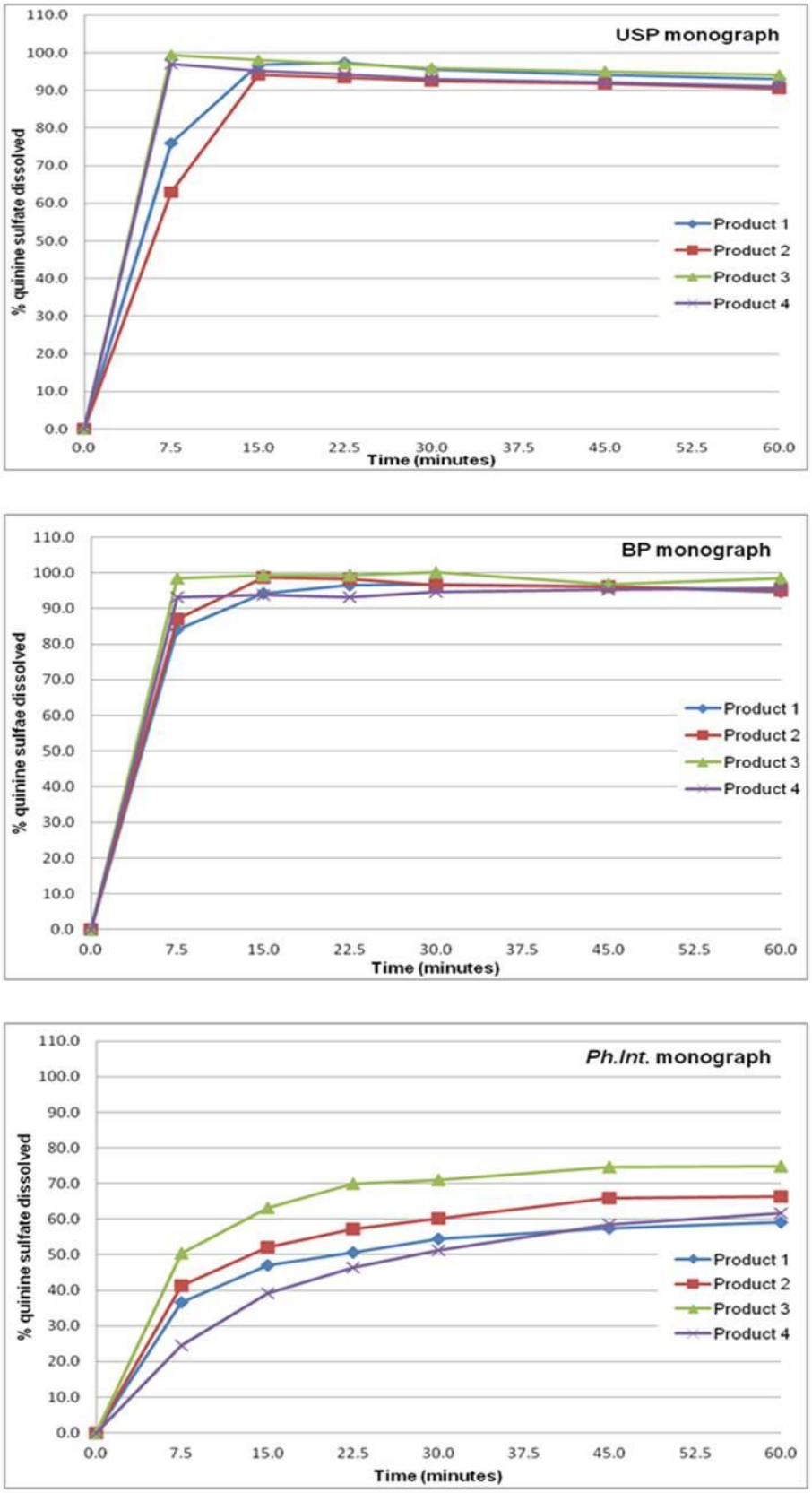

Figure 7-2: The dissolution profiles of the USP, BP and *Ph.Int.* quinine sulfate tablet monographs

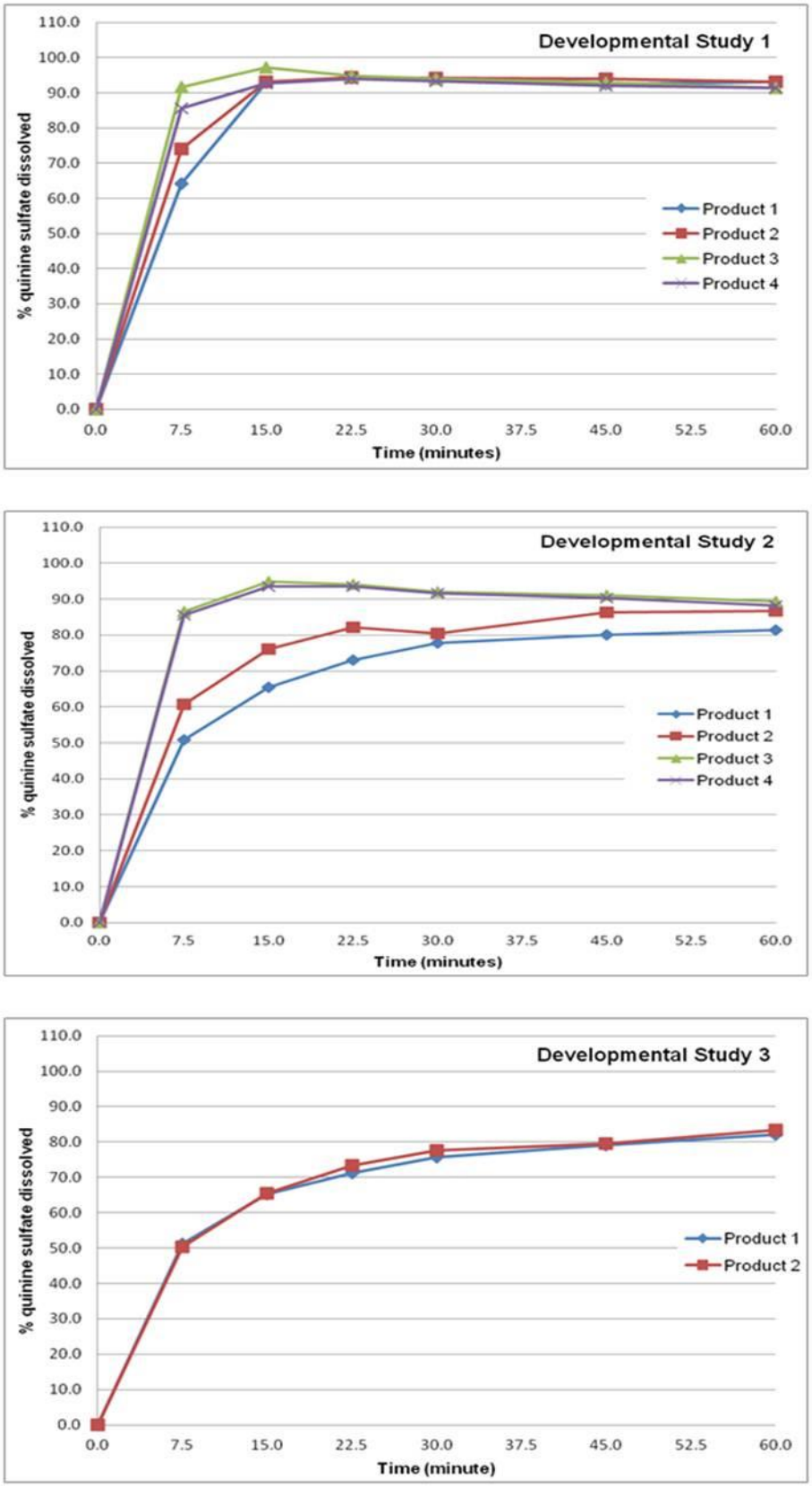

Figure 7-3: The dissolution profiles of Developmental study 1,2 and 3.

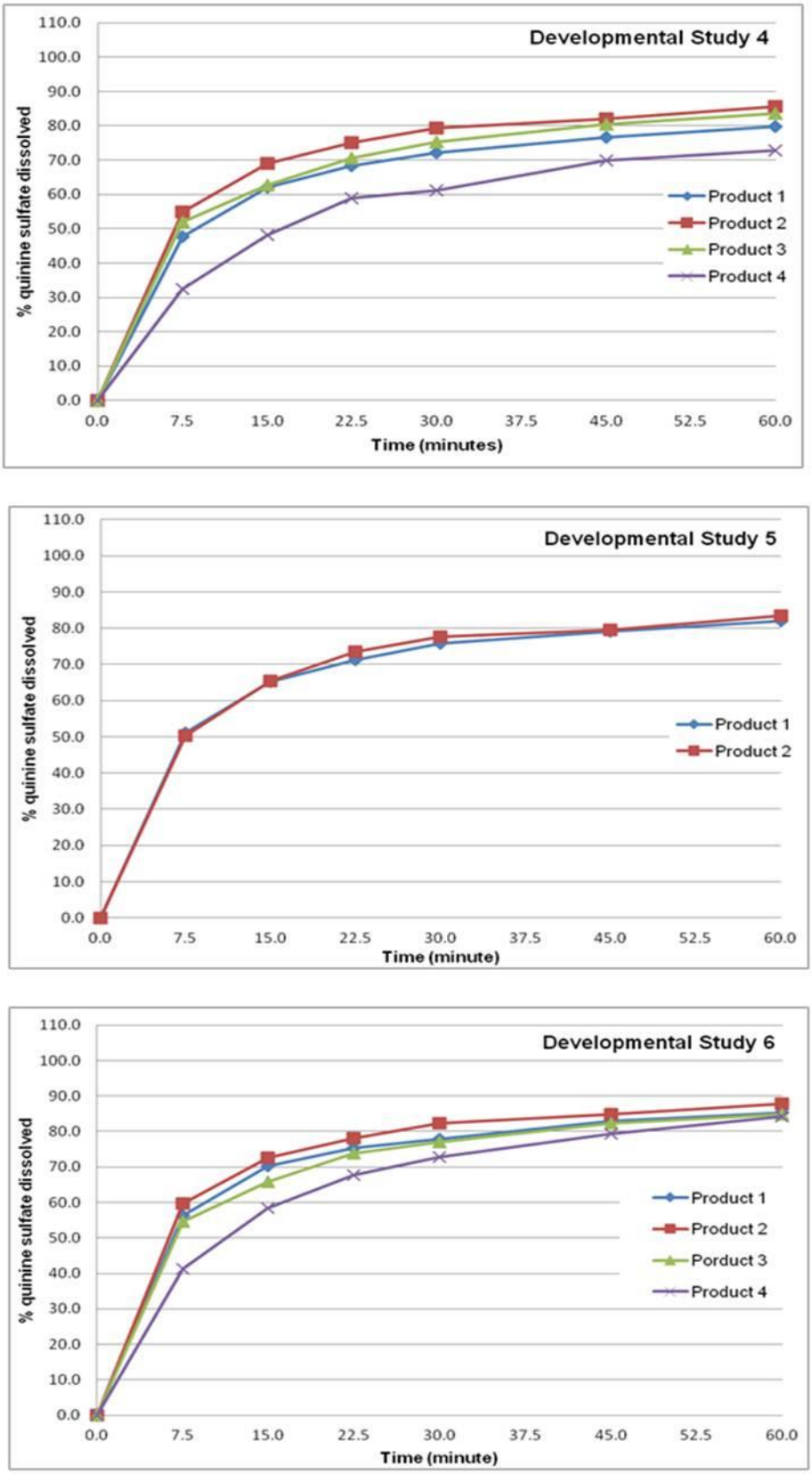

Figure 7-4: The dissolution profiles of the Developmental study 4, 5 and 6.

The results from Developmental study 6 showed the most promise in providing results that are repeatable, accurate, discriminatory, yet fair and for this reason the parameters of Developmental study 6 is proposed for consideration as an alternative dissolution method.

The results from this study found:

- that despite the differences in- and combinations of the identification tests used by the different pharmacopoeias, all pharmacopoeias identification tests were deemed adequately specific in identifying quinine sulfate in quinine sulfate tablets;
- limited statistical difference in the assay results obtained from quantification techniques (titration vs. HPLC) between the monographs, rendering the assay methods equal to present corresponding outcomes;
- corresponding outcomes between semi-quantitative (TLC) and quantitative (HPLC) related substances testing prescribed by the different pharmacopoeias;
- that indiscretion existed between the outcome of dissolution and disintegration tests intra-pharmacopoeial (*Ph.Int.* – choice between dissolution and disintegration);
- that indiscretion existed between the dissolution outcomes inter-pharmacopoeias;
    - which was followed by an in-depth investigation and developmental work;
        - which culminated in the proposal of an alternative dissolution test that allowed for fair yet discriminatory final outcomes.

By obtaining above mentioned results, the aims and objectives of this study were addressed providing a satisfying conclusion to this work.

> ***"Quality is never an accident. It is always the result of intelligent effort. There must be the will to produce a superior thing."***
>
> **—** *John Ruskin*