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Short Communication

Pharmaceutical formulation analysis using room temperature phosphorescence

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Introduction

The large-scale production of tablets and capsules requires the presence of a variety of additives to complement the active ingredient, enhance the physical appearance of the dosage form, improve the stability of the formulation, or to assist the drug's biological distribution [1]. These supposedly inert additives must be considered when assay procedures are established for the quality control of manufactured formulations. The widespread availability of generic brands of drugs, formulated using a variety of diluents, binders, lubricants, disintegrants and other adjuvants, requires that specific procedures be developed for the quantitation of active ingredients in dosage forms. Current official procedures for analysing drugs in pharmaceutical formulations can be rather cumbersome [2]. As a result, pharmaceutical manufacturers are evaluating high-performance liquid chromatography and spectroscopic methods such as luminescence techniques, as selective alternative methods of analysis for quality control. In this report, room temperature phosphorescence (RTP) is examined for the analysis of a variety of drugs in pharmaceutical preparations. While room temperature phosphorescence has been shown to be analytically useful, real sample applications have been limited [3, 4]. RTP is at least as sensitive as absorption spectrophotometry, but its greater spectral selectivity implies that physical separations are not always necessary.

Experimental

Reagents and materials

Drug standards and commercial preparations are summarized in Tables 1 and 2. Pharmaceutical preparations were purchased through normal commercial sources. All other reagents were of analytical grade.

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Table 1
Drugs studied and experimental conditions

Compound*	Solvent system†	Nominal wavelengths‡ ($\lambda_{ex}/\lambda_{em}$, nm)
Acetylsalicylic acid	5	325/445
p-Aminobenzoic acid	1	295/435
Aminophylline	2	275/435
Caffeine	2	275/435
Chloroquine	1	340/465
Dyphylline	2	275/435
Hydrochlorothiazide	4§	305/440
Naphazoline	2	290/490
Oxtriphylline	2	275/435
Propranolol	3	305/490
Reserpine	4	300/455
Salicylamide	5	325/445
Theobromine	2	275/435
Theophylline	2	275/435

* Hydrochlorothiazide, naphazoline and reserpine were gifts of Ciba-Geigy; oxtriphylline was a gift of Warner-Lambert; propranolol was a gift of Ayerst. All other compounds were purchased from Sigma Chemical Co. (St Louis, MO, USA).
† Solvent systems [7, 8] consisted of:
(1) water,
(2) aqueous 1 M KI,
(3) ethanol–aqueous 1 M KI (50:50 v/v),
(4) ethanol–aqueous 1 M KI + 1 M NaOH (50:50 v/v),
(5) System (4) except ethanol–aqueous system (2:98 v/v),
‡ Wavelength accuracy was ±2 nm.
§ RTP not analytically useful.

Apparatus

RTP measurements were made with an Aminco-Bowman spectrophotofluorimeter (SLM-Aminco, Urbana, IL, USA), fitted with a 150-W xenon arc lamp (901C, Conrad-Hanovia, Newark, NJ, USA), a laboratory-constructed phosphoroscope with a chopping rate of 200 Hz [5] for bar-RTP [6] and a variable gain photomultiplier tube (1P21, Hamamatsu, Middlesex, NJ, USA). An Aminco ratio-photometer supplied high voltage to the photomultiplier tube in addition to serving as a dc amplifier.

Standard and sample preparation

Stock solutions (200–400 μg/ml) were prepared by dissolving accurately weighed portions of standards in an appropriate solvent system (Table 1). Standard solutions were prepared daily by dilution of the stock solutions. The solvent systems employed were those previously used [7, 8] and which gave the largest phosphorescence signal-to-background ratios. For the analysis of commercial preparations (Table 2), samples were prepared for assay by dissolution or dilution with an appropriate solvent system. For the analysis of representative samples 20 tablets, or the contents of 20 capsules, were weighed and mixed with a pestle and mortar, and four portions were each dissolved in 10.0 ml of the appropriate solvent system to give solutions of nominally 100 μg/ml. For the analysis of liquid preparations, four appropriate aliquots of the samples were each diluted with the relevant solvent system to give final concentrations of 100 μg/ml of active ingredient.

Table 2
Sources of commercial preparations

Commercial description	Active ingredient	Source
Accurbron™*	Theophylline	Dow
Aminophyllin	Aminophylline	Searle
Anacin®	Acetylsalicylic acid	Whitehall
Aralen®	Chloroquine	Winthrop
Asbron G® Inlay-tabs®	Theophylline	Dorsey
Athemol®	Theobromine magnesium oleate	Glaxo
Bayer® aspirin	Acetylsalicylic acid	Glenbrook
BC® powder	Acetylsalicylic acid	BC Remedy
Bronkotabs®	Theophylline	Breon
Bufferin®	Acetylsalicylic acid	Bristol-Myers
Choledyl®	Oxtriphylline	Warner-Lambert
Elixophyllin® Elixir*	Theophylline	Berlex
Empirin®	Acetylsalicylic acid	Burroughs Wellcome
Excedrin™ Extra Strength	Acetylsalicylic acid	Bristol-Myers
Inderal® 20, 30, 80	Propranolol	Ayerst
Inderal® l*	Propranolol	Ayerst
Inderide® 40/25, 80/25	Propranolol/hydrochlorothiazide	Ayerst
Lufyllin-GG®	Dyphylline	Wallace
NoDoz®	Caffeine	Bristol-Myers
Pabanol™*	*p*-Aminobenzoic acid	P. B. Elder
Privine®*	Naphazoline	Ciba-Geigy
Quadrinal™	Theophylline calcium salicylate	Knoll
Serpasil®	Reserpine	Ciba-Geigy
Slo-Phyllin®-80 syrup*	Theophylline	Dooner
Slo-Phyllin®-60 Gyrocaps	Theophylline	Dooner
Theo-Dur®	Theophylline	Key
Vivarin®	Caffeine	J. B. Williams
Wyeth® aspirin	Acetylsalicylic acid	Wyeth

* Liquid preparations.

Procedure

For the bar-RTP analysis, an aluminium bar and its cover plate with four holes (each *ca* 6.25 mm dia.) and filter paper discs (grade 903, Schleicher & Schuell, Keene, NH, USA) were used. The filter paper discs were placed under the cover plate, which was tightened into place on the bar. Samples were spotted on to the paper discs in 5 μl volumes using a micro-pipette (Scientific Manufacturing Industries, Emeryville, CA, USA). The bar was then placed in the sample compartment, where the samples were allowed to dry for 7 min under a flow of dried nitrogen gas (*ca* 20 l/min). During the drying process, signal levels increased over a period of *ca* 6 min at which time a plateau was reached for *ca* 2 min. Measurements were made on this plateau using the excitation and emission wavelengths given in Table 1. This procedure was repeated 16 times (about 1 h was required in all) for each sample and standard. Quantitation was achieved using the RTP intensities of standards run concurrently. The range of linearity was established and linear regression analysis was performed for each set of data.

Results and Discussion

In this report, the selection of formulations to be analysed was made by consulting the *American Drug Index* [9]. For each generic type of drug, a variety of preparations were selected, in order to represent different formulation matrices, so that the selectivity

advantage of room temperature phosphorescence as a method of analysis of complex mixtures could be examined.

In the first study (Table 3), aminophylline, dyphylline, oxtriphylline and theophylline

Table 3
Determination of xanthines in pharmaceutical formulations

Sample*	Percentage recovery relative to label claim†		RSD(%)‡
	Mean	Range	
Aminophyllin[1]	100	92–111	5.8
Asbron G® Inlay-Tabs[4]	98	96–102	2.1
Bronkotabs®[4]	95	91–100	2.8
Choledyl®[3]	94	81–112	8.8
Lufyllin®-GG[2]	95	87–102	6.5
Quadrinal™[4]	94	91–99	2.8
Slo-Phyllin® 60 Gyrocaps®[4]	95	88–101	4.4
Theo-Dur®[4]	102	94–109	4.5
Slo-Phyllin®-80 syrup[4]	107	102–112	3.0
Elixophyllin® Elixir[4]	98	91–103	3.6
Accurbron™[4]	117	111–124	3.1

* All test samples comprised 100 μg/ml active ingredient, except Slo-Phyllin®-80 and Accurbron™ (107 μg/ml active ingredient).

† Calculated from 16 measurements of each of four samples using the corresponding standard calibration curve: Linear Dynamic Ranges: 25–300 μg/ml (y = relative phosphorescence signal, and x = analyte concentration, in μg/ml). [1]Aminophylline, $y = 0.43x + 0.37$; [2]dyphylline, $y = 0.1x + 1.5$; [3]oxtriphylline, $y = 0.42x + 0.55$; [4]theophylline, $y = 0.60x + 0.56$. All correlation coefficients ($p = 0.95$): $r > 0.9995$.

‡ RSD = relative standard deviation (%).

were analysed in various pharmaceutical formulations, including capsules, compressed tablets, enteric-coated tablets, elixirs and syrups. With the exception of Slo-Phyllin® and Accurbron™, the results agreed quite well with U.S.P. formulation analysis guidelines, which generally dictate 90–110% recovery of the active ingredient in pharmaceutical formulations, although for some formulations the limits are different, e.g. aspirin (95–105%) and aminophylline (93–107%). Precision studies with 16 determinations of each sample gave relative standard deviation (RSD) values between 2 and 9%. The proposed RTP procedure is simple and specific for the determination of several xanthines in the pharmaceutical preparations tested.

For the analysis of acetylsalicylic acid (ASA) in several analgesic preparations, the results in Table 4 were obtained. With the exception of the results for BC® powder, good recoveries were observed, with RSD between 2.4 and 4.4%. BC® powder contains both ASA and salicylamide. The latter phosphoresces at similar wavelengths to ASA and thus interferes in ASA determinations. In this study, the analysis of BC® powder was performed using only ASA standards: no correction factor was applied to compensate for the differences in phosphorescence sensitivities between ASA and salicylamide. Consequently, the results overestimated the amount of ASA in the powder, illustrating

Table 4
Determination of acetylsalicylic acid (ASA) in analgesic preparations

Preparation	Content of ASA (mg per dosage form)*	Percentage recovery relative to label claim†		RSD(%)
		Mean	Range	
Anacin®	400	103	97–109	3.2
Bayer® aspirin	325	101	96–106	3.4
BC® powder	650	138	134–142	2.4
Bufferin®	324	98	89–103	4.2
Empirin®	325	94	87–101	3.9
Excedrin®	250	101	92–106	4.4
Wyeth® aspirin	300	101	92–106	3.7

* Amount of ASA claimed on label. All test samples contained nominally 100 μg/ml ASA. In addition, the test sample of BC® powder contained 30 μg/ml salicylamide, since BC® powder contains both ASA (650 mg) and salicylamide (195 mg) per dose.

† Calculated from 16 measurements of each of four samples, using the standard calibration curve. Linear dynamic range: 10–200 μg/ml ASA; $y = 16.5x + 59$. Correlation coefficient ($p = 0.95$) $r > 0.9995$.

the type of problem encountered when more than one component can phosphoresce.

The determination of propranolol in Inderal® and Inderide® formulations gave good recoveries (Table 5), with RSD between 4.0 and 7.2%. Hydrochlorothiazide, present in Inderide® formulations, was not assayed because its phosphorescence was too weak.

To evaluate further the use of RTP in real sample analyses, caffeine, chloroquine, naphazoline, *p*-aminobenzoic acid (PABA), reserpine and theobromine were assayed in a variety of pharmaceutical formulations. The method yielded good recoveries (Table 6), with RSD values in the range 1.9–5.2%.

Table 5
Determination of propranolol in pharmaceutical formulations

Preparation and content of active ingredient (mg per dosage form)*	Percentage recovery relative to label claim†		RSD(%)
	Mean	Range	
Inderal®			
20	98	90–108	5.2
40	97	88–108	7.2
80	102	94–108	4.0
1	103	94–111	4.4
Inderide®			
40/25	102	94–110	5.0
80/25	101	94–110	4.5

* All test samples contained nominally 100 μg/ml active ingredient. Inderal® 1 is an injectable ampoule containing 1 mg in 1 ml. Inderide® contains propranolol/hydrochlorothiazide in the amounts (mg) indicated.

† Calculated from 16 measurements of each of four samples, using the standard calibration curve. Linear dynamic range: 20–300 μg/ml. Propranolol: $y = 0.725x - 7.34$. Correlation coefficient ($p = 0.95$): $r > 0.9995$.

Table 6
The determination of various active ingredients in pharmaceutical formulations

Preparation	Content of active ingredient*	Percentage recovery relative to label claim†		RSD(%)
		Mean	Range	
Aralen®[2]	300	102	94–108	4.7
Athemol®[6]	74	103	100–106	1.9
NoDoz®[1]	100	99	89–108	5.2
Pabanol™[4]	5% m/v	101	95–105	3.6
Privine®[3]	0.05% m/v	108	101–116	4.3
Serpasil®[5]	0.5	98	98–103	2.8
Vivarin®[1]	200	100	91–108	5.0

* Expressed as mg per dosage form, or concentration as % m/v. For Athemol® the equivalent theobromine weight in theobromine magnesium oleate is given. Others are listed as amount of active ingredient. All test samples comprised 100 μg/ml active ingredient.

† Calculated from 16 measurements of each of four samples, using the standard calibration curve.

Compound	Linear dynamic range	Regression data
[1]Caffeine	25–200 μg/ml	$y = 6.1\,x - 5.6$
[2]Chloroquine	25–200 μg/ml	$y = 9.5\,x + 6.5$
[3]Naphazoline	25–200 μg/ml	$y = 11.1\,x + 1.9$
[4]*p*-Aminobenzoic acid	1–200 μg/ml	$y = 29\,x - 10.1$
[5]Reserpine	25–300 μg/ml	$y = 1.3\,x + 2.7$
[6]Theobromine	25–200 μg/ml	$y = 2.6\,x + 7.0$

All correlation coefficients ($p = 0.95$): $r > 09995$.

The results show that room temperature phosphorescence is worth consideration for the selective determination of certain drugs in pharmaceutical formulations. Future work should be directed towards improving the precision of measurement by way of a thorough study of the sources of variability. Instrumental performance could be improved by better optimization of the solvent system to give increased signal intensity, by use of an image detector [10] and by automation of the mode of sample presentation [11]. The use of time-resolved phosphorimetry to discriminate between the analyte signal and interference due to other formulation components or to background may further extend the selectivity of RTP in pharmaceutical analysis [12].

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