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Simultaneous multiwavelength spectrophotometric quantitation of active components in analgesic formulations. Comparative study of three calculation methods*

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Abstract: This work deals with the UV spectrophotometric quantitation of a mixture of compounds with overlapped spectra. The mixture spectrum is resolved by use of three computational programs based on different algorithms, namely Multicomponent Analysis (commercial software available from Hewlett–Packard), MULTIC (relying on multiple regression analysis) and SIMPLEX. The results obtained for mixtures of acetylsalicylic acid, acetaminophen and caffeine in commercial analgesic formulations, are compared.

Keywords: *Multicomponent analysis; acetylsalicylic acid; acetaminophen; caffeine; analgesic analysis; UV spectrophotometry.*

Introduction

UV-visible spectrophotometry is an instrumental technique of wide use in pharmaceutical analysis on account of its rapidity, simplicity and applicability to a host of pharmacologically active species and their derivatives absorbing in that spectral zone. The quantitation method traditionally used involves measuring the analyte absorbance at a given wavelength. However, the analyte of interest is often accompanied by other compounds absorbing in the same spectral region. Such cases require resolution of the spectral overlap by using masking agents or separating the different components of the mixture. Thus, HPLC with spectrophotometric detection has become one of the analytical techniques most frequently used in pharmaceutical analysis.

Simultaneous determinations are interesting alternatives to specific determinations insofar as they result in simpler, more easily automated analytical procedures [1, 2].

In spite of the fact that UV spectrophotometric multicomponent analysis has been available for a relatively long time [3], only recently i.e. ever since computers have

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become popular and affordable, has its application grown to significant levels. The concentrations of the different components of the mixture have been calculated by using several procedures based on the least-squares method or by adapting iterative sequential methods such as the SIMPLEX or Kalman's filter [4–8]. However, when these calculation procedures are applied to real samples, they do not all afford the same accuracy and no *a priori* prediction can be *made* in this respect. The selection of the mathematical program thus becomes an important step for the accurate determination of a multicomponent mixture.

The accuracy and precision of the results obtained also relies on the wavelength range used and the absorbance ratios between the different components of the mixture [2, 9, 10].

The aim of this work was to compare three calculation programs based on very different algorithms.

These programs have been applied to resolution of prepared mixtures of acetylsalicylic acid, acetaminophen and caffeine and to their quantitation in two commercial formulations.

Computational programs used in the mixture quantitation

Mixture spectra were resolved with the aid of three computational programs: Multicomponent Analysis (MA), SIMPLEX and MULTIC.

Assuming that the matrix effect and chemical interactions are negligible, the form of Beer's law corresponding to the mixture of N species absorbing at j wavelengths is

$$A_j = \sum_{i=1}^{i=N} \varepsilon_{i,j} b\, c_i$$

where: A_j and $\varepsilon_{i,j}$ are the mixture absorbance and absorptivity of species i, respectively, at each wavelength; b is the path length; and c_i is the concentration of species i.

All three programs use spectra from pure standards of accurately known concentrations as reference and yield the analyte concentrations resulting in the best fit between the calculated and experimental spectra.

Multicomponent analysis

This is part of the Hewlett–Packard spectrophotometer bundled software. It is written in machine code, so that its algorithm is unknown to the user. The program fits a combination of standard spectra of the components to the mixed spectrum by the least-squares method. Agreement between experimental and calculated spectra is given by a fit error parameter.

SIMPLEX

This is the well-known iterative optimisation program [11] whose algorithm has been adapted for multicomponent analysis. The program calculates the concentrations minimizing the function $U = \Sigma(A_{exp} - A_{cal})^2$ at all wavelengths. The fit accuracy is given by the U value and by the standard deviation.

MULTIC

This program performs multiple regression of the sample absorbance hyperplane in a space of $N + 1$ dimensions (for N components). The fit between calculated and experimental spectra is given by the standard deviation and correlation coefficient. The program also provides the independent term (intercept on the absorbance axis).

Experimental

Reagents

Standard solutions were prepared by weighing and dilution in 15% (v/v) MeOH:H_2O acetylsalicylic acid (ASA) and caffeine (CAF) (Fluka purum) acetaminophen (AAP), citric acid (CIT) and salicylic acid (SA) (Carlo Erba RPE).

Working solutions were prepared by appropriate dilution in a phosphate buffer of pH 7 containing 15% (v/v) MeOH:H_2O. The final phosphate concentration was always 5×10^{-3}M.

Fiorinal™ capsules were obtained from Sandoz SAE (Spain) and Actron™ tables were supplied by Miles Martin Laboratorios SAE (Spain).

Apparatus

All absorption spectra were acquired on a Hewlett–Packard HP-8451A diode array spectrophotometer furnished with a quartz cell of 10 mm path length and equipped with an HP-9121 floppy disc drive for bulk data storage and an HP-7470A digital plotter for graphic presentation of data.

The spectrophotometer bundled software was used for the mathematical treatment of the spectra and for mixture resolution by the MA program. Spectra were transferred to and manipulated by an IBM PC-XT microcomputer for SIMPLEX and MULTIC routines.

Procedure

The reliability of the mixture resolution depends chiefly on the accuracy of the spectral data of pure components used as reference in resolving the mixture. Three solutions of concentration falling in the linear range and giving absorbance readings in the range 0.6–1.2 were prepared for each component. The three spectra recorded for each solution were averaged out into one which was normalised at a concentration of 10^{-5}M (10^{-3}M in the case of CIT). Finally, the normalised spectra corresponding to the three solutions were also averaged out to obtain a single spectrum which was used as standard (Fig. 1).

The spectra of the mixtures and samples were obtained by averaging out three spectra from single solutions.

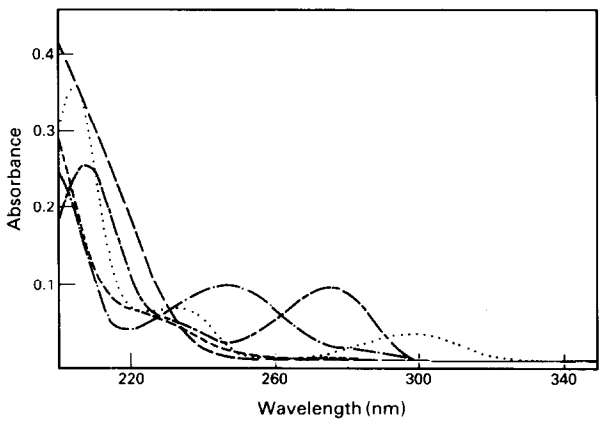

Figure 1
Standard spectra of: 10^{-5}M ASA (- - -); 10^{-5}M AAP (– · – ·); 10^{-5}M CAF (– – –); 10^{-3}M CIT (— — —); 10^{-5}M SA (· · · ·).

All spectra were obtained at an integration time of 10 s. In analysing the active components of Fiorinal, the contents of 10 capsules were mixed and three aliquots of about 300 mg were treated with 15 ml MeOH, made to 100 ml with bidistilled water and filtered. The active components of Actron were analysed by grinding 5 tablets and three aliquots of about 200 mg were dissolved in about 20 ml water with continuous stirring. Once degassed, 15 ml of MeOH were added and stirring continued until dissolution was complete, after which the solution was made to 100 ml. Suitable dilution was affected in both cases, and enough phosphate buffer was added to ensure a final composition of 15% (v/v) MeOH:H_2O and 5×10^{-3}M phosphate, pH 7 and a maximum absorbance less than 1.

Results and Discussion

The results obtained by any procedure reported in the literature are usually satisfactory provided all components contribute similarly to the mixture absorbance. However, significant errors occur when components contributing to a much lesser extent than the otheres are determined. Since this is rather a common situation in the analysis of pharmaceutical formulations, a reliable computational method is required in such cases.

Table 1
Relative error (%) in the determination of ASA, AAP and CAF in prepared mixtures

	M.A.	SIMPLEX	MULTIC
Ratio 10:5:1			
ASA 2.58×10^{-5}M	−0.73	−0.85	−0.69
APP 1.33×10^{-5}M	2.11	2.18	1.80
CAF 2.56×10^{-6}M	2.66	1.95	0.47
f.e.:	3.5	*U*: 1.27×10^{-5}	*I.T.*: 4.76×10^{-4}
		S: 5.43×10^{-4}	*S*: 5.13×10^{-4}
			R: 0.99999
Ratio 5:5:1			
ASA 1.30×10^{-5}M	1.65	1.57	1.26
APP 1.33×10^{-5}M	0.56	0.49	0.79
CAF 2.61×10^{-6}M	1.23	1.92	3.03
f.e.:	11.42	*U*: 7.29×10^{-6}	*I.T.*: 3.77×10^{-4}
		S: 4.12×10^{-4}	*S*: 3.87×10^{-4}
			R: 0.99999
Ratio 5:10:1			
ASA 1.28×10^{-5}M	−0.19	−0.19	1.84
APP 2.65×10^{-5}M	2.72	2.75	1.70
CAF 2.58×10^{-6}M	12.83	12.37	4.55
f.e.:	112.6	*U*: 8.46×10^{-5}	*I.T.*: 2.59×10^{-3}
		S: 1.40×10^{-3}	*S*: 9.33×10^{-4}
			R: 0.99997
Ratio 10:10:1			
ASA 1.25×10^{-5}M	0.94	0.78	0.62
APP 1.27×10^{-5}M	0.48	0.40	0.55
CAF 1.30×10^{-6}M	−0.97	0.27	1.33
f.e.:	15.45	*U*: 3.84×10^{-6}	*I.T.*: -2.19×10^{-4}
		S: 2.99×10^{-4}	*S*: 2.89×10^{-4}
			R: 0.99999

f.e.: fit error; *U*: minimisation function; *S*: standard deviation; *I.T.*: independent term; *R*: regression coefficient.

With this idea in mind, the programs compared in this work were applied to the resolution of mixtures of three components (ASA, AAP and CAF), one of which was present at a much lower concentration than the other two.

Earlier work [2] demonstrated that the best result in the resolution of mixtures of species with overlapped spectra are obtained when the widest possible wavelength range is used. Such a range was 210–300 nm throughout this work.

The results obtained in the resolution of prepared mixtures of ASA, AAP and CAF in molar ratios of about 10:5.1, 5:10:1, 5:5:1 and 10:10:1 are listed in Table 1.

All three components were determined highly accurately by the three programs. Only in one instance, namely the 5:10:1 mixture, were significant errors observed in the determination of caffeine, particularly by the MA and SIMPLEX methods. The fit parameters of all three programs and the intercept provided by the MULTIC were significantly worse for this mixture than for the others, which can be attributed to the weak contribution to the spectrum of this component. This effect is corrected by MULTIC program (through the independent term), and consequently better results are obtained.

Determination of ASA, AAP and CAF in commercial formulations

Fiorinal is available in capsules and Actron in effervescent tablets with nominal contents of 200 mg ASA, 300 mg AAP, 40 mg CAF and excp. — not stated — the former, and 267 mg ASA, 133 mg AAP, 40 mg CAF, 954 mg CIT and 1,606 mg $NaHCO_3$ the latter. In Figs 2 and 3 are shown the spectra of these active principles at their nominal analytical concentrations and the corresponding overall spectrum. Note the small contribution of CIT at wavelengths above 210 nm.

These formulations represent two different situations; in Fiorinal solution, the absorbing species are only the active principles while in ACTRON a weakly absorbing excipient remains in solution.

The results obtained in Fiorinal and ACTRON analysis are listed in Tables 2 and 3 respectively.

As can be seen, the precision of all three computational programs in Fiorinal analysis was similar and the results were always close to the stated nominal values. Addition of

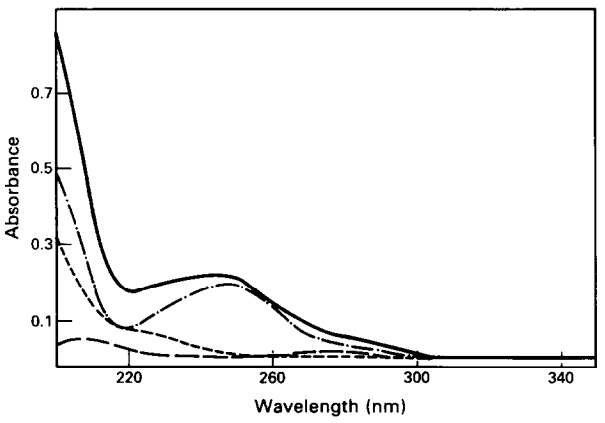

Figure 2
Spectra of the mixture (———); ASA (- - -); AAP (– · – ·) and CAF (— — —) at the nominal analytical concentrations in Fiorinal.

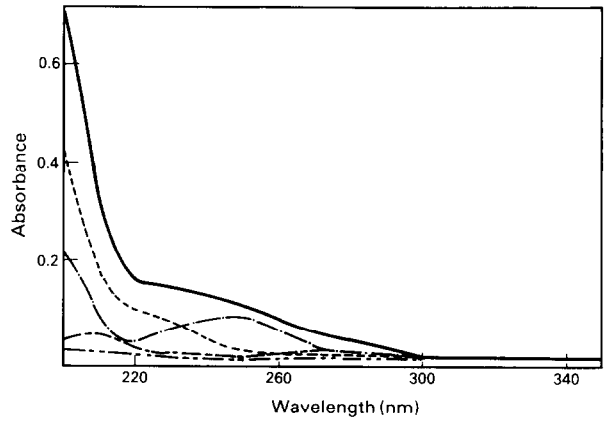

Figure 3
Spectra of the mixture (———); ASA (- - -); AAP (- · - ·); CAF (— - — -) and CIT (— — —) at the nominal analytical concentrations in ACTRON.

SA to the mixture resulted in better fit parameters, the AAP and CAF concentrations found were not significantly changed, and were very similar for all three programs. Conversely, considerable variations were found in the ASA content while ASA + SA content remained virtually constant.

Because of the hydrolysis of ASA, accurate quantitation of the mixture required performing the analysis immediately after sample dissolution (Table 4). Yet, if only AAP and CAF were to be determined, their quantitation could be safely postponed as they are not affected by the presence of appreciable amounts of SA.

Four models were tested in the resolution of the ACTRON spectrum. In these models the sample absorbance was attributed to: (a) only the active principles: ASA, AAP and CAF; (b) the four nominal components: ASA, AAP, CAF and CIT; (c) the active principles plus SA; (d) the active principles plus SA and CIT.

If it is assumed that the best model for the mixture spectra resolution is the one that shows best fit between calculated and experimental spectra, then, from data in Table 4 it is evident that model d is the most appropriate for ACTRON quantitation.

Very similar results with MA and MULTIC were found when the three calculation programs were applied to this model, while SIMPLEX produced significant differences. In all cases the imprecision of CIT was large and its value itself not reliable, which can be explained by the very small contribution of CIT to the mixture absorbance.

The high precision of the determination of AAP and CAF with MULTIC should be noted.

Conclusions

The three computational programs used allow the quantitation of substances with overlapped spectra in mixtures when the contributions of components to the composite spectra are disparate with similar rapidity, accuracy and precision. In principle, the MULTIC program algorithm which includes an independent term seem to be preferable.

The accurate resolution of a mixture generally requires the prior knowledge of the components actually present and of their contribution to the mixture absorbance. The

Table 2. Determination of active components in Fiorinal. Results (mg per capsule) expressed as the average and maximum deviation in the analysis of three aliquots of a mixture of ten capsules

	MA	SIMPLEX	MULTIC
ASA	212.1 ± 2.8	212.0 ± 3.1	215.5 ± 3.0
AAP	311.7 ± 10.1	311.8 ± 10.3	308.6 ± 9.4
CAF	43.5 ± 1.1	43.8 ± 0.8	40.4 ± 1.3
	fe.: 101.2 ± 29.5	*U*: $(6.5 \pm 1.4)10^{-5}$	*I.T.*: $(2.2 \pm 0.7)10^{-3}$
		S: $(1.2 \pm 0.15)10^{-3}$	*S*: $(8.03 \pm 1.4)10^{-4}$
			R: 0.99997 ± 0.0002
ASA	199.1 ± 3.1	203.1 ± 2.0	210.1 ± 5.5
AAP	311.7 ± 10.1	311.8 ± 10.4	309.1 ± 9.1
CAF	42.3 ± 0.9	42.5 ± 0.9	41.5 ± 1.0
SA	8.3 ± 1.1	6.8 ± 0.4	5.5 ± 1.0
	fe.: 27.0 ± 16.6	*U*: $(3.43 \pm 2.35)10^{-5}$	*I.T.*: $(1.90 \pm 0.77)10^{-3}$
		S: $(8.78 \pm 2.92)10^{-4}$	*S*: $(4.23 \pm 0.66)10^{-4}$
			R: 0.99998*

*$R > 0.99999$ in the three determinations. Other symbols as in Table 1. Label claim: ASA: 200 mg; AAP: 300 mg; CAF: 40 mg per capsule.

Table 3. Determination of active components in ACTRON. Results (mg per tablet) expressed as the average and maximum deviation in the analysis of three aliquots of a mixture of five tablets

	MA	SIMPLEX	MULTIC
ASA	307.4 ± 1.7	314.6 ± 3.9	312.8 ± 1.5
AAP	130.5 ± 4.1	128.1 ± 5.1	129.8 ± 1.0
CAF	49.0 ± 4.8	52.0 ± 4.1	53.5 ± 2.0
	fe.: 1160.1 ± 210.9	*U*: $(9.4 \pm 3.7)10^{-4}$	*I.T.*: $(-0.96 \pm 2.55)10^{-3}$
		S: $(4.61 \pm 0.99)10^{-3}$	*S*: $(4.59 \pm 0.98)10^{-3}$
			R: 0.9978 ± 0.0008
ASA	289.9 ± 11.9	276.2 ± 13.4	274.9 ± 11.0
AAP	133 ± 2.7	134.6 ± 2.6	134.3 ± 0.3
CAF	48.3 ± 5	49.4 ± 4.5	48.6 ± 1.3
CIT	794 ± 591	1718.4 ± 772.7	1817.4 ± 545.1
	fe.: 1300 ± 255	*U*: $(8.77 \pm 3.43)10^{-4}$	*I.T.*: $(3.1 \pm 2.6)10^{-3}$
		S: $(4.51 \pm 0.91)10^{-3}$	*S*: $(3.1 \pm 2.6)10^{-3}$
			R: 0.9980 ± 0.00082
ASA	270.1 ± 4.9	258.6 ± 6.1	249.4 ± 15.3
AAP	130.7 ± 4.1	128.4 ± 5.1	134.1 ± 1.1
CAF	40.7 ± 4.1	45.5 ± 4.4	50.3 ± 1.6
SA	26.0 ± 3.3	33.3 ± 5.9	35.2 ± 7.8
	fe.: 363.3 ± 134.3	*U*: $(2.05 \pm 1.3)10^{-4}$	*I.T.*: $(-3.02 \pm 2.89)10^{-3}$
		S: $(2.12 \pm 0.76)10^{-3}$	*S*: $(1.57 \pm 0.23)10^{-3}$
			R: 0.99974 ± 0.00013
ASA	197.6 ± 21.3	173.2 ± 11.0	195.4 ± 22.5
AAP	138.7 ± 3.0	141.7 ± 6.9	140.3 ± 1.4
CAF	41.0 ± 4.4	49.4 ± 4.5	43.5 ± 0.3
CIT	2637 ± 677	3381.4 ± 291.5	2475.3 ± 629.6
SA	33.9 ± 4.6	38.3 ± 2.1	36.4 ± 8.0
	fe.: 76.2 ± 104.4	*U*: $(7.71 \pm 6.59)10^{-5}$	*I.T.*: $(-1.35 \pm 2.9)10^{-3}$
		S: $(1.27 \pm 0.75)10^{-3}$	*S*: $(0.99 \pm 1.52)10^{-3}$
			R: 0.99999*

*$R > 0.99999$ in the three determinations. Other symbols as in Table 1. Label claim: ASA: 267 mg; AAP: 133 mg; CAF: 40 mg; CIT: 954 mg per tablet.

Table 4
Effect of hydrolysis in Fiorinal analysis. The results are expressed in mg per capsule

	M.A.		SIMPLEX		MULTIC	
	(1)	(2)	(1)	(2)	(1)	(2)
ASA	215.6	98.9	214.9	103.1	223.0	106.0
AAP	306.8	306.1	307.1	306.0	304.9	304.1
CAF	41.9	42.8	41.6	43.4	40.2	41.8
SA	0.1	88.5	—	85.6	—	85.0

(1) 1 h and (2) 24 h after the sample dissolution.

occurrence of a component scarcely contributing to the overall absorbance may result in significant deviations.

It should be emphasised that very good fits between experimental and calculated spectra do not necessarily reflect the accurate resolution of the mixture.

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References

[1] A. van Loosbroeck, H. J. G. Debets and P. M. J. Coenegrarcht, *Anal. Lett.* **17(B9)**, 779–792 (1984).
[2] M. Blanco, J. Gené, H. Iturriaga and S. Maspoch, *Analyst* **112**, 619–622 (1987).
[3] J. C. Sternberg, H. S. Stillo and R. H. Schwendeman, *Anal. Chem.* **32**, 84–90 (1960).
[4] D. J. Leggett, *Anal. Chem.* **49**, 276–281 (1977).
[5] J. A. Howell and L. G. Hargis, *Anal. Chem.* **58**, 108R–124R (1986).
[6] L. S. Ramos, K. R. Beebe, W. P. Carey, E. Sánchez, B. C. Erikson, B. E. Wilson, L. E. Wangen and B. R. Kowalski, *Anal. Chem.* **58**, 294R–315R (1986)
[7] S. D. Brown, *Anal. Chim. Acta* **181**, 1–26 (1986).
[8] K. R. Beebe and B. R. Kowalski, *Anal. Chem.* **59**, 1007A–1017A (1987).
[9] D. P. Rossi and H. L. Pardue, *Anal. Chim. Acta* **175**, 153–161 (1985).
[10] S. D. Frans and J. M. Harris, *Anal. Chem.* **57**, 2680–2684 (1986).
[11] S. N. Deming and S. L. Morgan, *Anal. Chem.* **45**, 278A–282A (1973).

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