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An Innovative Method, Interference Subtraction, for Determination of Drugs in Syrups in Presence of Potential Interfering Excipients

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Abstract: Pharmaceutical dosage forms contain both active ingredients and inactive materials called exipients, most of which cause high interference specially in direct UV-spectrophotometry. This problem is highly obvious in syrups which may contain preservatives, coloring agents, thickening agents and sweetening agents. Thus, there is an urgent need to develop and validate a simple, accurate and selective method named interference subtraction method for determination of drugs in syrups in presence of these excipients without pre-separation by solving this interference. This newly designed method was successfully applied to; Oxybutynin hydrochloride in Detronin® syrup, Ambroxol hydrochloride in Muco® syrup and Ibuprofen in both Brufen® syrup and Megafen N® oral suspension. These chosen syrups contain different excipients causing different types of interference. This innovative method was validated according to ICH guidelines. The results obtained by interference subtraction method were statistically compared with those obtained by the official and /or reported methods of the proposed drugs.

Key words: Interference, subtraction , oxybutynin, ambroxol, ibuprofen.

Introduction

The interference subtraction method is proposed to solve the interference caused by different exipients in syrups and is established by application to four different syrups containing different active ingredients and also different types of exipients as shown by the different types and extents of interference they cause in the zero order UV-absorption spectra .

The used syrups contain oxybutynin hydrochloride, ambroxol hydrochloride and ibuprofen.

The literature review shows different methods for determination of oxybutynin hydrochloride in syrups including; model updated PLS chemometrics and HPTLC [1] and HPLC methods [2,3].

Ambroxol hydrochloride was determined in syrups by diffuse reflectance spectroscopy [4], HPLC [5], Sequential Injection Chromatography [6].

The British pharmacopoeia determined ibuprofen in syrups by an HPLC method [7]. Ibuprofen in syrups was also determined by spectrofluorimetry [8].

Principle

For each drug, a calibration curve is constructed relating the absorbance of pure drug at selected λ to the concentration of the drug then the regression equation is computed (1).

The absorbance of every concentration of pure drug at the selected λ is subtracted from the absorbance of syrup containing the same concentration of drug along with the interfering excipients. This will give the contribution of excipients in the syrup spectra.

Another calibration curve is constructed relating this contribution of excipients in the syrup spec-

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tra to the concentration of drug in the syrup using different dilutions of syrup ,then the equation was obtained from the constructed curve (2).

Say you want to determine concentration X of the drug in a certain syrup dilution:

i) Record the absorbance of the sample under test at the selected λ.

ii) Find the contribution of the excipients by substitution by the predicted concentration in equation (2).

iii) Subtract step (ii) from step (i) to obtain the absorbance of the drug at the selected λ.

iv) Apply in equation (1) to obtain the concentration of the drug in the syrup, and then calculate its recovery.

Experimental

Samples

Pure samples

Pure standard oxybutynin HCl and ambroxol HCl were kindly supplied by the Egyptian Co. for chemicals and pharmaceuticals, ADWIA, 10th of Ramadan city-A.R.E, with certified purity of 99.84 %.

Pure standard Ibuprofen was kindly supplied from 10th of Ramadan for Pharmaceutical Industries & Diagnostic Reagents (rameda) 6th of October city-A.R.E., with certified purity of 99.56 %.

Market samples

Detronin® syrup manufactured by Pharaonia Pharmaceuticals Pharo Pharma Company for Pharmaceutical Industries. New Borg El-Arab city, Alexandria – A.R.E., labeled to contain 100 mg of oxybutynin HCl for each 100 mL syrup. Three different batches were used (Batch No. 4035001-4034002-4033006).

Muco® syryp manufactured by Global Napi Pharmaceuticals. 2nd Industrial Zone, 6th of October city-A.R.E., labeled to contain 15 mg of ambroxol HCl for each 5 mL syrup. Three different batches were used (Batch No. 1446026-1446007-1446021).

Megafen N® oral suspension manufactured by 10th of Ramadan for Pharmaceutical Industries & Diagnostic Reagents (rameda) 6th of October city-A.R.E., labeled to contain 100 mg of Ibuprofen for each 5 mL suspension. Two different batches were used (Batch No. 141141-141326).

Brufen® syrup manufactured by Kahira Pharmaceuticals & Chemical Industries company-Egypt, labeled to contain 100 mg of Ibuprofen for each 5 mL syrup. Two different batches were used (Batch No. 49088/3j-41994/3j).

Chemicals and reagents

Methanol HPLC grade

Prepared solutions

Stock standard solutions (1 mg/mL) of oxybutynin HCl, ambroxol HCl and Ibuprofen were prepared by accurately weighing and dissolving 100 mg of each drug in 100 mL methanol in a separate 100 mL flask.

Working standard solutions (0.1 mg/mL) of oxybutynin HCl, ambroxol HCl and Ibuprofen were prepared by transferring 10 mL of each stock solution into a separate 100 mL flask and completing to the marks with methanol.

Syrup solutions (0.1 mg/mL)

For Detronin® syrup, 10 ml of the syrup were transferred into a 100 mL volumetric flask. About 75 mL of methanol was added and the flask was completed to the mark with methanol.

For Muco® syrup, 16.67 ml of the syrup were transferred into a 50 mL volumetric flask. Completed to the mark with methanol to obtain a stock solution of 1 mg/mL. From the prepared stock solution, transfer 10 mL to a 100 mL volumetric flask and complete to the mark with methanol.

For Megafen N® oral suspension and Brufen® syrup, 2.5 mL from each were transferred into a 50 mL volumetric flask, completed to the mark with methanol to obtain a stock solution of 1 mg/mL. From the prepared stock solution, transfer 10 mL to a 100 mL volumetric flask and complete to the mark with methanol.

Instruments

A Double beam UV-VIS spectrophotometer (Shimadzu , Kyoto- Japan), model UV-1601 PC with 1 cm quartz cells, connected to IBM compatible computer. The bundled software, UV-PC personal spectroscopy software version 3.7 was used, the spectral band is 2 nm and scanning speed is 2800 nm / min with 0.1 nm interval.

Procedures

Spectral characteristics

The absorption spectra of pure oxybutynin HCl and Detronin® syrup solutions each containing 10 µg/mL of oxybutynin HCl were recorded over the wavelength range of 200 - 400nm using methanol as blank, Figure 1.

The absorption spectra of pure Ambroxol HCl and Muco® syrup solutions each containing 12 µg/mL of Ambroxol HCl were recorded over the wavelength range of 200 - 400 nm using methanol as blank, Figure 2.

The absorption spectra of pure Ibuprofen and Brufen® syrup solutions each containing 10 µg/mL of Ibuprofen were recorded over the wavelength range of 200 - 400 nm using methanol as blank, Figure 3.

The absorption spectra of pure Ibuprofen and Megafen N® oral suspension each containing 10 µg/mL of Ibuprofen were recorded over the wavelength range of 200 - 400 nm using methanol as blank, Figure 4.

Construction of calibration curves

For pure drugs

Aliquots equivalent to (6-24) µg/mL of oxybutynin HCl are accurately transferred from its standard working solution (0.1 mg/mL) to a series of 10 mL volumetric flasks, and then completed to volume with methanol. The spectra of the prepared solutions are scanned from 200-400. A calibration curve relating the absorbance at 215 nm to the corresponding concentration in µg/mL of oxybutynin HCl is constructed.

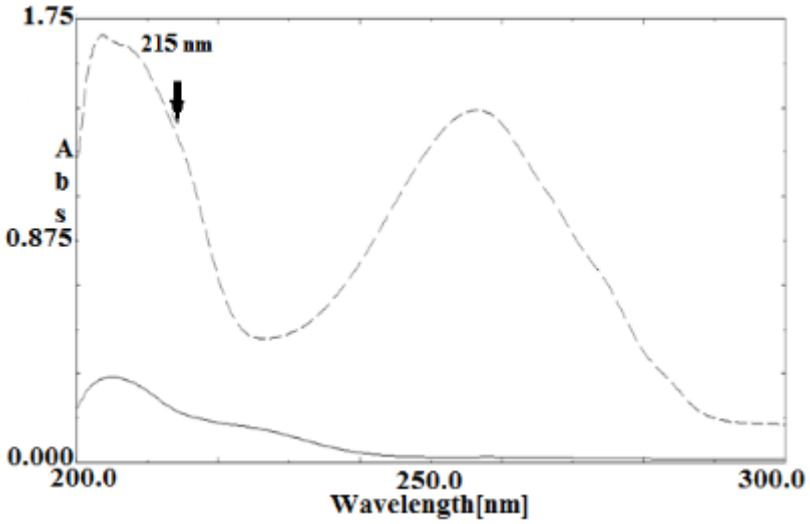

Figure 1. The UV absorption spectra of pure oxybutynin 10 µg/mL (solid line) and Detronin® syrup 10 µg/mL (dashed line)

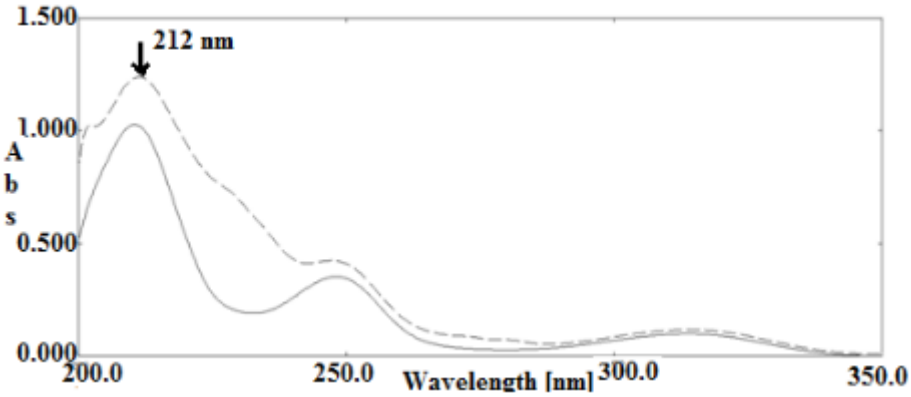

Figure 2. The UV absorption spectra of pure Ambroxol HCl 12 µg/mL (solid line) and Muco® syrup 12 µg/mL (dashed line)

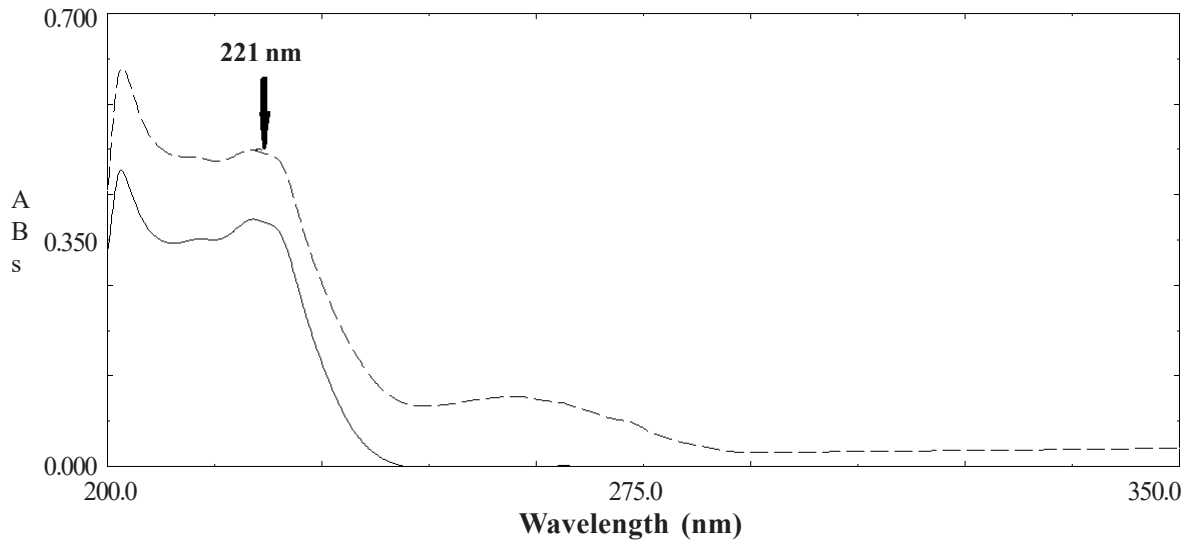

Figure 3. The UV absorption spectra of pure Ibuprufen 10 µg/mL (solid line) and Brufen® syrup 10 µg/mL (dashed line)

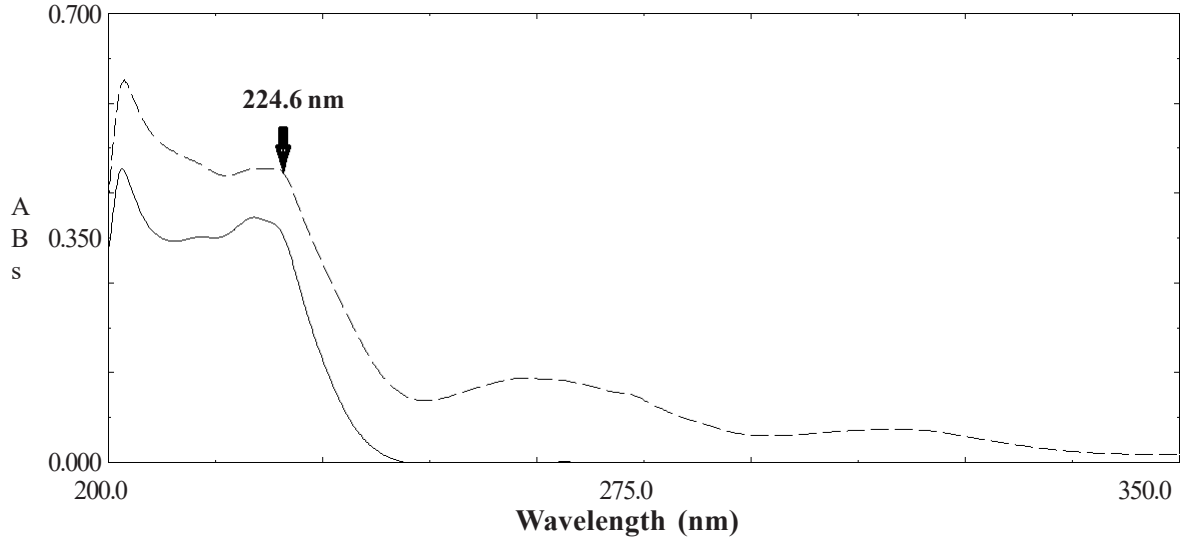

Figure 4. The UV absorption spectra of pure Ibuprufen 10 µg/mL (solid line) and Megafen N® suspension 10 µg/mL (dashed line)

For the determination of ambroxol HCl, aliquots equivalent to (4-19) µg/mL are accurately transferred from its standard working solution (0.1 mg/mL) to a series of 10 mL volumetric flasks, and then completed to volume with methanol. The spectra of the prepared solutions are scanned from 200-400. A calibration curve relating the absorbance at 212 nm to the corresponding concentration in µg/mL is constructed.

For the determination of Ibuprofen, aliquots equivalent to (4-22) and (5-22) µg/mL are accurately transferred from its standard working solution (0.1 mg/mL) to 2 separate series of 10 mL volumetric flasks, and then completed to volume with methanol. The spectra of the prepared solutions are scanned from 200-400. Two calibration curves relating the absorbance at 221 and 224.6 nm respectively to the corresponding concentration in µg/mL are constructed.

For interferent contribution

Aliquots equivalent to (6-18, 9-19, 6-20, 10-20) µg/mL of oxybutynin HCl , ambroxol HCl and Ibuprofen are accurately transferred from the standard working solutions (0.1 mg/mL) of Detronine® syrup , Muco® syrup , Brufen® syrup

and Megafen N® suspension to four separate series of 10 mL volumetric flasks, and then completed to volume with methanol. The spectra of the prepared solutions are scanned from 200-400. Four calibration curves relating the contribution of interfering exipient (absorbance difference) at 215, 212, 221 and 224.6 nm, for the four dosage forms, respectively, to the corresponding active ingredient concentration in µg/mL.

Results and discussion

An exipient is defined as a material that has been evaluated for safety that aids in the manufacture of the dosage form, and protects, supports,or enhances the stability and bioavailability of the active ingredient [9].

Each exipient has been selected for its functionality, and it has been deemed safe in the particular application and compatible with other components of the formulation.

Many of these exipients cause severe overlapping interference with the zero order absorbance of the active ingredients especially in syrups and suspensions, which obligates the analysts to use chromatographic methods to analyze this type of dosage forms as clearly shown in the literature review. The chromatographic methods are known to be more time consuming, more tedious, more expensive and less convenient than zero order spectrophotometric methods.

The focus of this work is to enable the analysts to use zero order spectrophotometry to analyze syrups and oral suspensions that suffer severe interference from the exipients, by designing and developping a new spectrophotometric method (Interference subtraction method) capable of solving this severe overlapping interference.

Method development

Interference subtraction method starts by scanning zero order spectra of pure drugs as well as the dosage forms of the same concentrations, followed by recording the absorbance of each of them at the selected wavelength.

The absorbance of each concentration of the pure drug at the selected wavelength is then subtracted from the absorbance of the same concentration of the dosage form at the same wavelength giving the contribution of the exipients in the absorbance of the dosage form.

A calibration is constructed relating the absorbance of the pure drug at the selected wavelength to its concentration in µg/mL. The regression equation is then computed. (equation 1).

The calibration curve of the exipient contribution is constructed by plotting the exipient contribution (absorbance difference) in each dosage form to its active ingredient concentration in µg/mL. The regression equation is then computed. (equation 2).

To obtain the absorbance of the active ingredient in the dosage form, the exipient contribution obtained from equation 2 is subtracted from the absorbance of the syrup at the selected wavelength.

The concentration of the active ingredient in the dosage form is obtained by compensating by its absorbance (obtained in the previous step) in equation 1. The percentage recovery is then calculated.

Method optimization

Several wavelengths were tried, where the selected wavelengths were those at which the pure drug has considerable absorbance and the exipients have an overlapping interference. This choice provides the best linearity for eq 1 and eq 2.

The number of batches used in the construction of eq 2 is also an important factor. One to three batches were used to construct eq 2, and it was found that; using three batches for each syrup gives the best results regarding accuracy.

Different concentration ranges were tried for construction of eq 1 and eq 2, where the selected ranges help to achieve the best linearity represented by suitable slope and intercept values.

Application to syrups

The method was successfully applied to; Detronin® syrup, Muco® syrup, Prufen® syrup and Megafen N® suspension.

In all of these syrups the exipients cause severe overlapping interference with the active ingredient as obviously shown in figures 1-4.

The calibration curves relating the absorbances

of pure oxybutynin HCl , ambroxol HCl and Ibuprofen at thier corresponding wavelengths to their concentrations in µg/mL are shown in figures 5-8.

Their regression equations parameters are shown in table 1.

The calibration curves relating the exipient contributions in Detronine® syrup, Muco® syrup, Brufen® syrup and Megafen N® suspension to their active ingredients concentrations in µg/mL are shown in figures 9-12. Their regression equations parameters are shown in table 2.

Method validation

Linearity and range

Good linearity was evident by the high value of the correlation coefficient and the low intercept value, as shown in tables 1 & 2.

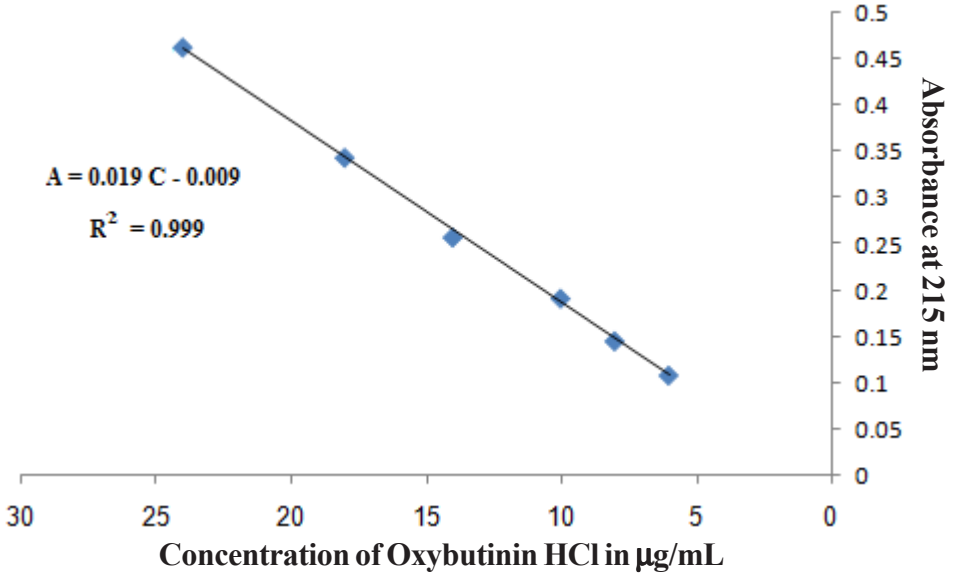

Figure 5. Calibration curve relating the absorbance at 215 nm to the concentration of oxybutinin HCl in µg/mL

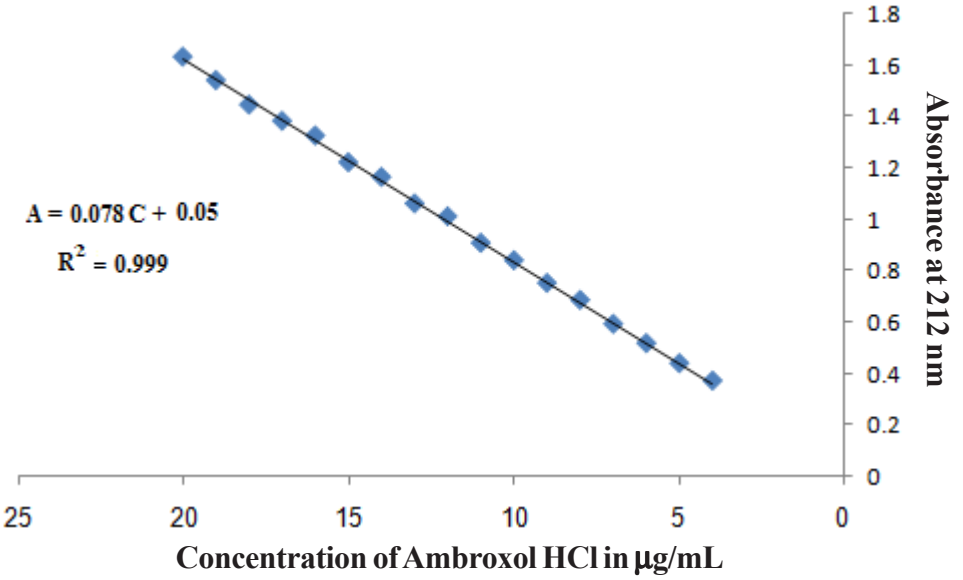

Figure 6. Calibration curve relating the absorbance at 212 nm to the concentration of ambroxol HCl in µg/mL

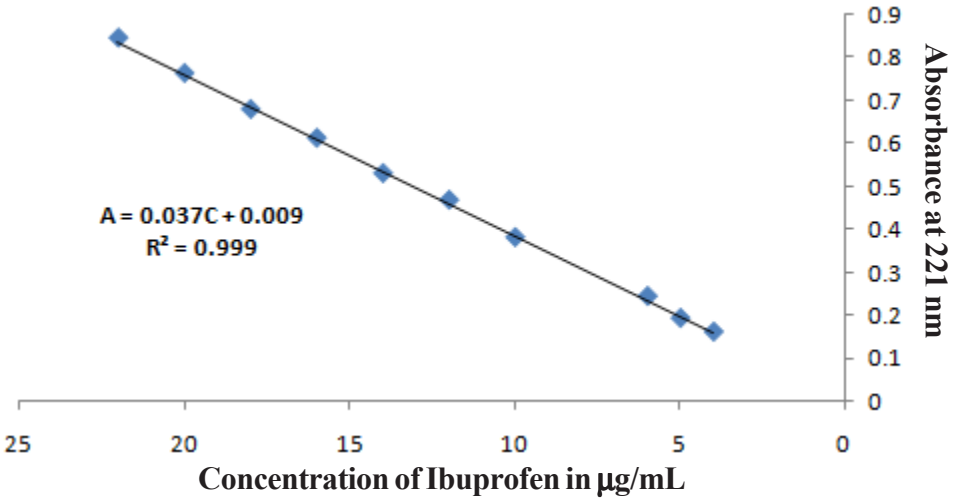

Figure 7. Calibration curve relating the absorbance at 221 nm to the concentration of Ibuprofen in µg/mL

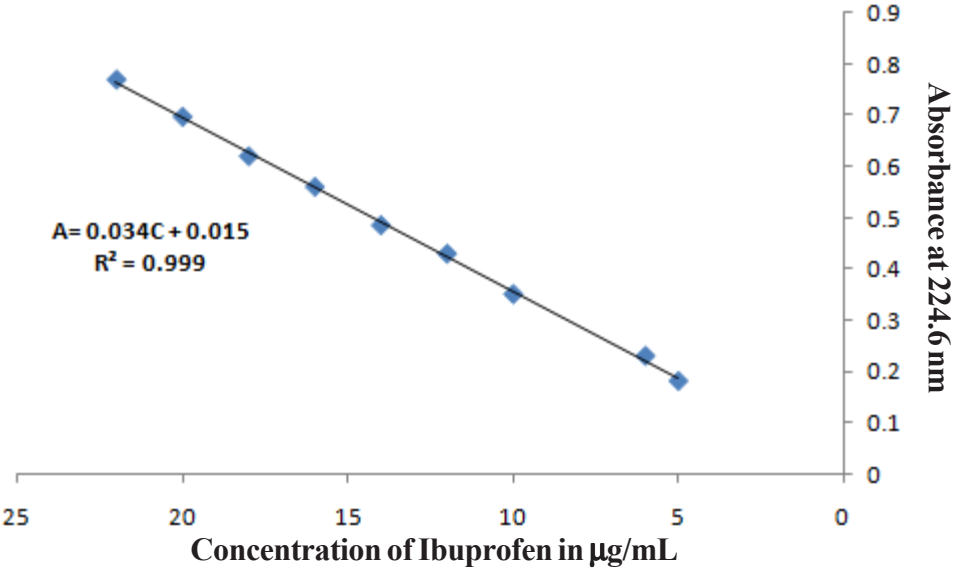

Figure 8. Calibration curve relating the absorbance at 224.6 nm to the concentration of Ibuprofen in µg/mL

Table 1. The regression equation parameters for pure Oxybutinin HCl at 215 nm, Ambroxol HCl at 212 nm, Ibuprufen at 221 nm and 224.6 nm

Parameters	Oxybutinin HCl at 215 nm	Ambroxol HCl at 212nm	Ibuprufen at 221 nm	Ibuprufen at 224.6 nm
Calibration range	6-24	4-19	4-22	5-22
Slope	0.0196	0.0785	0.0376	0.0341
Intercept	-0.0095	0.0500	0.0095	0.0156
Correlation coefficient	0.9995	0.9996	0.9996	0.9995
Mean±SD	100.14±1.765	100.06±1.174	100.18±1.795	100.08±2.261

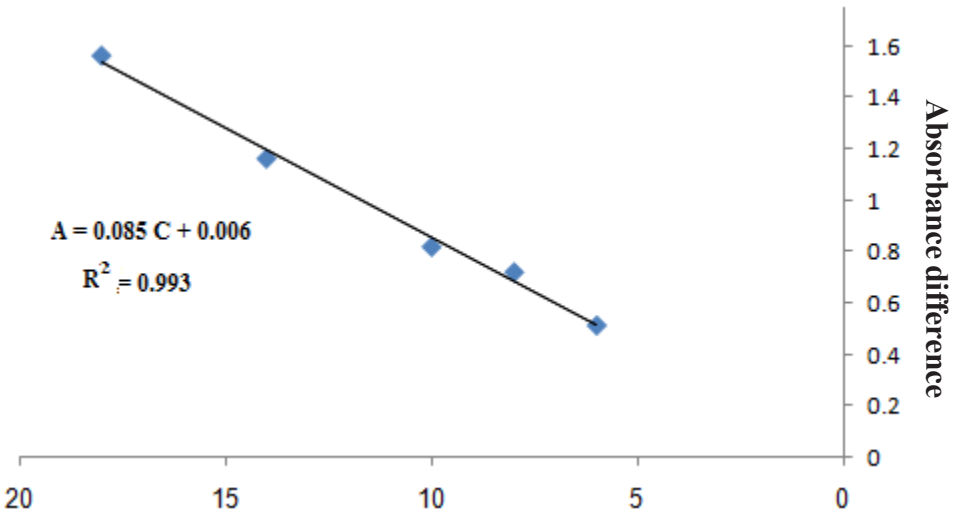

Figure 9. Calibration curve relating the interferent contribution in detronine® syrup to the active constituent concentration

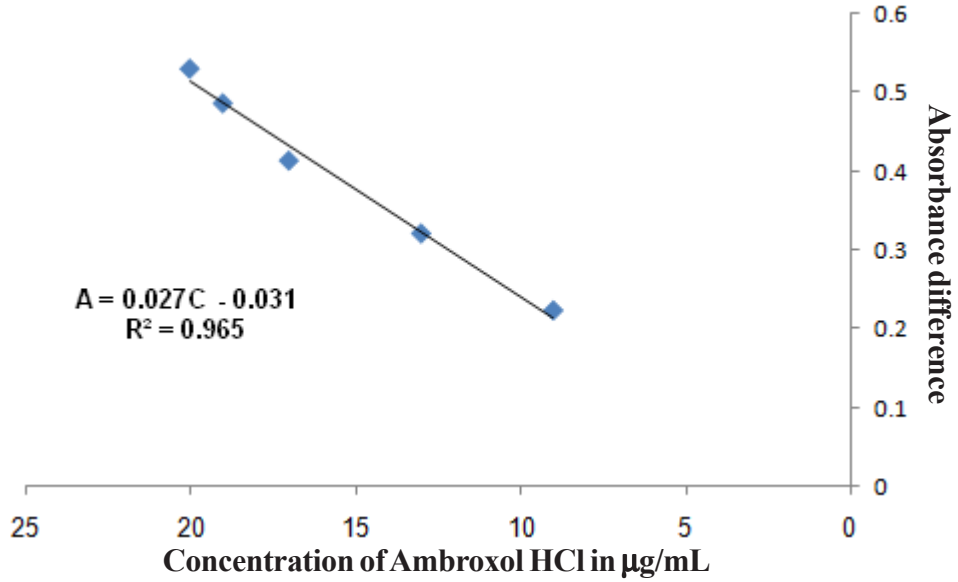

Figure 10. Calibration curve relating the interferent contribution in Muco® syrup to the active constituent concentration

Table 2. The regression equation parameters for exipient contribution (absorbance difference) in Detronin® syrup, Muco® syrup Megafen N® oral suspension and Brufen® syrup

Parameters	Detronine syrup	Muco syrup	Brufen syrup	Megafen N suspension
Calibration range	6-18	9-19	6-20	10-20
Slope	0.0850	0.0270	0.0144	0.0158
Intercept	0.006	-0.031	-0.0576	-0.068

table 2 (continued).

Parameters		Detronine syrup	Muco syrup	Brufen syrup	Megafen N suspension
Correlation coefficient		0.9968	0.9825	0.9933	0.9944
Precision	Repeatability	0.332	0.298	0.423	0.213
	Intermediate precision	1.131	0.978	1.267	0.832
Mean±SD		100.19±3.784	101.16±5.212	102.96±5.669	105.43±2.949

[a] R.S.D.% and [b] R.S.D.% : the intra-day and inter-day relative standard deviations of concentrations (, and µg mL^{-1})

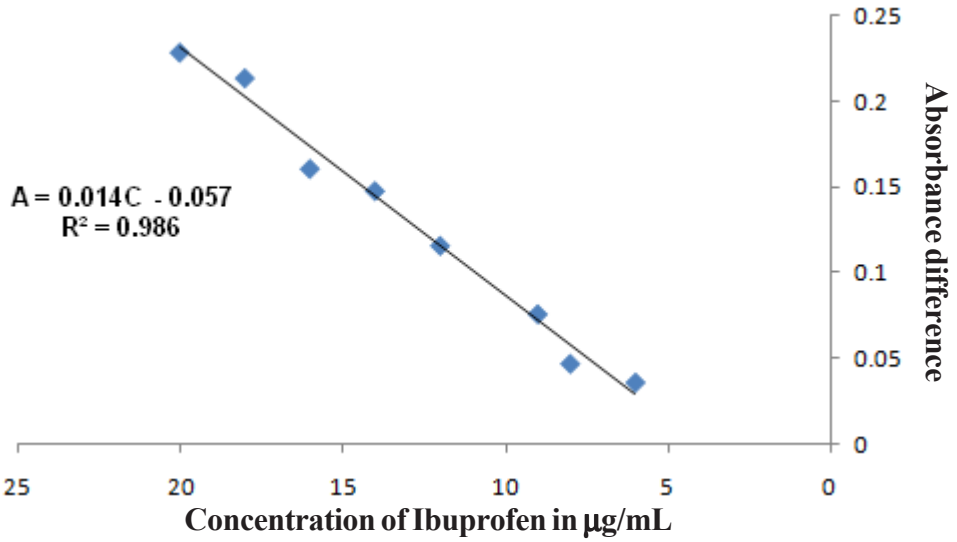

Figure 11. Calibration curve relating the interferent contribution in Prufen® syrup to the active constituent concentration

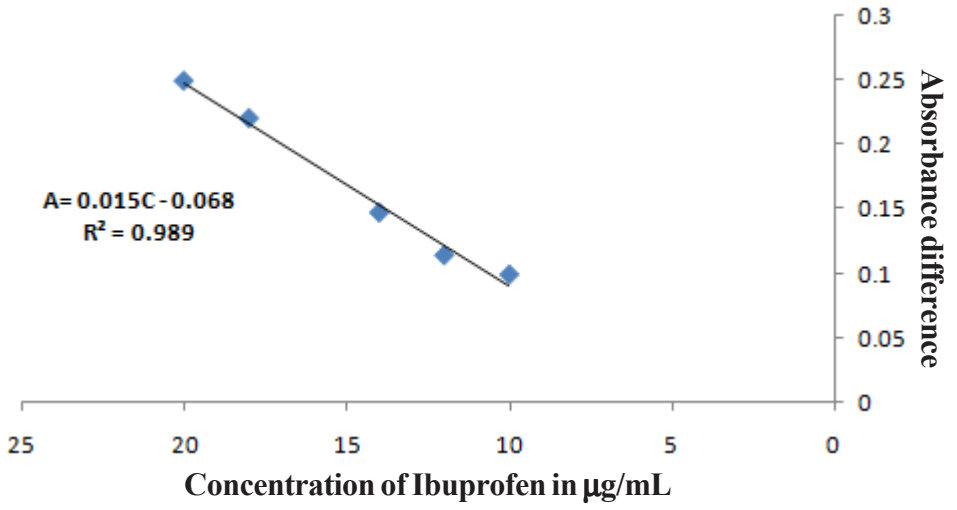

Figure 12. Calibration curve relating the interferent contribution in Megafen N® suspension to the active constituent concentration

Accuracy

The accuracy of the method was checked by applying the proposed method for determination of active ingredient concentrations in different batches of the proposed syrups, table 3-5. Accuracy was further assured by the use of the standard addition technique for Muco® syrup., table 4.

Precision

Repeatability

Repeatability of the results for concentrations (10, 13 and 17 µg mL^{-1}) of the studied drugs in the proposed syrups was performed by three replicate determinations to estimate intra-day variation, table 2.

Intermediate precision

The previous procedures were repeated for analysis of the concentrations (10, 20 and 30 µg mL^{-1}) (10, 13 and 17 µg mL^{-1}) of the studied drugs in the proposed syrups, three times on four different days, within the same week to estimate inter-day variation**,** table 2.

Selectivity

The method was successfully applied to different syrups which suffer different types of exipients interference as shown in their spectra in figures 1-4.

Good results obtained in tables 3-5 assured the method selectivity.

Table 3. Application of the interference subtraction method to Detronin® syrup

	Taken (µg mL^{-1})	Found* (µg mL^{-1})	Recovery %
Detronine® syrup	6*	5.95	99.12
	8***	8.42	105.26
	11*	10.74	97.61
	12**	12.89	107.46
	18***	19.21	106.73
Mean ± SD			103.24 ± 4.548

* Detronin® syrup, batch No. 4034002
** Detronin ® syrup, batch No. 4033006
*** Detronin ® syrup, batch No. 4035001

Table 4. Application of the interference subtraction method to Muco® syrup and application of standard addition technique

	Taken (µg mL^{-1})	Found* (µg mL^{-1})	Recovery %
Muco® syrup	11**	10.96	99.63
	12***	11.39	94.98
	18*	17.28	96.01
Mean ± SD			96.87±2.442
Standard addition	**Pure added (µg mL^{-1})**		**Recovery %**
	5		99.21
	6		100.91
	9		101.28
	10		102.07
Mean ± SD			100.87 ± 1.206

* Muco® syrup, batch No. 1446021
** Muco® syrup, batch No. 1446007
*** Muco® syrup, batch No. 1446026

Table 5. Application of the interference subtraction method to Brufen® syrup and Megafen N® oral suspension

	Taken (µg mL^{-1})	Found* (µg mL^{-1})	Recovery %
Brufen® syrup	3^	2.98	99.33
	5^	4.96	99.20
	9^^	8.54	94.89
	10^	10.01	100.10
	12^^	11.35	94.59
	15^	15.02	100.13
	17^^	16.19	95.23
Mean ± SD			97.64 ± 2.589
Megafen N® oral	10*	10.24	102.35
suspension	11*	11.24	102.14
	12**	11.76	98.04
	15**	14.59	97.25
	18*	18.35	101.96
	19**	18.26	96.13
	20*	20.71	103.52
Mean ± SD			100.20 ± 2.956

^ Brufen® syrup, batch No. 49088/3j

^^ Brufen® syrup, batch No. 41994/3j

* Megafen N® oral suspension , batch No. 141326

** Megafen N® oral suspension , batch No. 141141

Table 6. Statistical comparison between the interferent subtraction method and the reference methods for analysis of Detronin syrup and Muco syrup

Parameters	Detronin syrup ® *		Muco syrup ® **	
	Interferent subtraction	Reference method [a]	Interferent subtraction	Reference method [b]
Mean ± SD	97.60±1.503	98.33±1.772	99.63±1.566	99.42±1.517
Degree of freedom	10		10	
F-test (5.05)	1.390		1.065	
Student's t-test (2.228)	0.772		0.243	

* Equivalent to 11 µg/mL Oxybutinin HCl , batch No. 4034002

** Equivalent to 11 µg/mL Ambroxol HCl, batch No. 1446007

[a] An HPLC method for determination of Oxybutynin hydrochloride in presence of its degradation products [3]

[b] An HPLC method for the quantification of Ambroxol hydrochloride/benzoic acid in syrups [5]

Statistical analysis

The results obtained by the innovative interference subtraction method were compared with those obtained by the reported methods for oxybutynin hydrochloride [3], ambroxol hydrochloride [5] and ibuprofen [7].

The calculated t and F values were less than the theoretical ones indicating that there is no significant difference between the proposed and the reported methods regarding accuracy and precision as shown in tables 6,7.

Conclusion

The spectrophotometric methods are simple,

Table 7. Statistical comparison between the interferent subtraction method and the reference methods for analysis of Megafen N suspension and Brufen syrup

Parameters	Megafen N suspension ®*		Brufen syrup ®**	
	Interferent Subtraction	Reference Method[a]	Interferent Subtraction	Reference Method[b]
Mean ± SD	101.96±1.500	102.28±1.561	101.10±1.595	100.83±1.445
Degree of freedom	10		10	
F-test (5.05)	0.863		1.216	
Student's t-test (2.228)	0.365		0.305	

* Equivalent to 18 µg/mL Ibuprofen , batch No. 141326

** Equivalent to 10 µg/mL Ibuprofen, batch No. 49088/3j

[a] The British pharmacopoeial HPLC method for determination of ibuprofen in syrups [7]

more convenient, less time consuming and economic methods compared to LC methods. These advantages encouraged us to establish our method to improve the selectivity and remove the interference caused by different additives in syrups. The developed method is capable of determining the active constituents in syrups without prior separation.

References

1. **Wagieh, N.E., Hegazy, M.A., Abdelkawy, M., Abdelaleem, E.A. (2010).** Quantitative determination of oxybutynin hydrochloride by spectrophotometry, chemometry and HPTLC in presence of its degradation product and additives in different pharmaceutical dosage forms. Talanta (80): 2007-2015.
2. **De-Schutter, J.A., De-Moerloose, P. (1988).** Determination of oxybutynin chloride in pharmaceuticals by reversed-phase ion-pair liquid chromatography with two counter-ions in the eluent. J. Chromatogr. 450 (3): 337-342.
3. **El Gindy, A. (2005).** High performance liquid chromatographic determination of oxeladin citrate and oxybutynin hydrochloride and their degradation products. IL Farmaco. 60: 689.
4. **José L. Rufino, Joel M. Santos, Mayra A. Rodrigues, Helena R. Pezza, Leonardo Pezza. (2009).** Determination of ambroxol in syrups using diffuse reflectance spectroscopy. Quim. Nova, 32(6): 1513-1516.
5. **Heinanen, M. and Barbas, C. (2001).** Validation of an HPLC method for the quantification of ambroxol hydrochloride and benzoic acid in a syrup as pharmaceutical form stress test for stability evaluation. J. Pharm. Biomed. Anal. 24(5-6): 1005-1010,
6. **Satinsky, D., Huclova, J., Ferreira, R.L., Montenegro, M.C. and Solich, P. (2006).** Determination of ambroxol hydrochloride, methylparaben and benzoic acid in pharmaceutical preparations based on sequential injection technique coupled with monolithic column. J. Pharm. Biomed. Anal. 40(2): 287-293.
7. **The British Pharmacopoeia, (2007).** Her Majesty's Office, London.
8. **Damiani, P.C., Bearzotti, M., Cabezon, M.A. (2001).** Spectrofluorometric determination of ibuprofen in pharmaceutical formulations. J. Pharm. Biomed. Anal., 25(3-4): 679-683,
9. **Myra L. Weiner and Lois A. Kotkoskie; (1999).** Exipient Toxicity and Safety, 103rd Ed; 2.