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Comparative Study of Electrospray Mass Spectrometry and First Derivative Method and Validation by HPLC Method

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ABSTRACT

Three different methods are described for the simultaneous determination of amoxicillin trihydrate (AMX) and potassium klavulanate (KLV) in synthetic mixtures and pharmaceutical tablets. The first method depends on the first-derivative ultraviolet spectrophotometry with zero-crossing measurement. The second method is based on mass spectrometry utilizing electropspray ionization. For the electrospray studies in negative and positive ion modes, aspirin (ASP) and clindamycin (CLN) were used, respectively as internal standards for quantification. The calibration function was established in the same concentration ranges as in the first derivative method. A reversed-phase high performance liquid chromatographic (RP-HPLC) method involving ultraviolet detection (λ = 220 nm) was developed for the analysis of AMX and KLV. Chromatography was carried out on a C-18 column with mobile phase comprising of phosphate buffer-acetonitrile (40:60, v/v). The proposed methods have been validated with regard to the selectivity, detection limit, recovery, accuracy and precision. For both drugs, methods were found to be selective, linear ($R \approx 0.99$), accurate (recovery = 100-105%) and precise (<3% RSD) in the range of 21-49 μg/mL for AMX and 3-7 μg/mL for KLV. The limit-of-detection and limit-of-quantification of the method were determined for three methods.

Key words: amoxicillin, potassium klavulanate, first derivative, electrospray mass spectrometry, HPLC

INTRODUCTION

Analysis of multi-mixtures without separation is of big importance in pharmaceutical, food, forensic and other areas. Numerical methods called chemometric methods[1-6] are well known and widely used for the analysis of multi-component mixture without a prior separation. It is necessary to understand several abstract theories to employ the numerical methods and most of the chemists do not wish to go into the details of those methods. For easy and fast data manipulation, graphical methods, namely derivative methods[7-12], can be used for the overlapped spectra of mixtures. Although graphical methods provide no separation step for the mixtures, they need additional mathematical calculation, like derivation of each spectrum. Recently in pharmaceutical area, mass spectrometry became to be used often especially for the drug quantification purposes[13-18]. Mass spectrometry, especially combined with HPLC provides better quantitation. Using HPLC as a hyphenated technique front, mass spectrometry, requires mostly electrospray ionization source as the ionization technique. This provides very soft ionization with minimal fragmentation, which depends on the applied voltage in the ion source. Separation step is not required for mass spectrometric methods. Depending on the ionization method, there exists several ways to quantitate the subjected compounds in the mixtures. For example electron impact may cause a lot of fragmentation and thus makes calculations harder. However, with the relative abundance of fragments of ions, it is possible to quantitate the compounds in the gas phase. By softer ionization methods namely electrospray, it is possible to get better quantitation for a single compound or mixtures using an internal standard without any separation. Chromatographic methods especially HPLC, are used in the pharmaceutical field for the qualitative or quantitative studies. HPLC provides very satisfactory results as long as a very robust method was developed. The disadvantage of this method is high cost, extra work, time-consumption and sometimes it is incapable of separating components in the mixtures.

In this report, three methods have been developed for the quantitative resolution of AMX and KLV in the synthetic binary mixture or tablet formulations. The challenging ration in the tablets makes it really hard to quantitate these components in the pharmaceutical preparations by spectrophotometric methods. The proposed methods solve this problem and eliminate any negative effects such as overlapping spectra or separation of compounds. The spectrophotometry and chromatography are well known methods, but electrospray ionization mass spectrometric (ESI-MS) method is not completely realized in the quantitative pharmaceutical studies. The main purpose of this study is to develop spectrophtometric and ESI-MS methods, compare the results, discuss their advantage and shortcomings and validate both results by HPLC method.

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MATERIALS AND METHODS

I. *Instrumentation and Mobile Phase*

The absorption measurements were carried out on a Shimadzu UV-240 double beam UV-VIS spectrophotometer with a fixed slit width (1 nm) and Shimadzu UVPC software. Data processing, regressions and statistical analysis were performed by using the UVPC and EXCEL softwares.

ESI-MS measurements were performed on a Finnigan LCQ ion trap MS (San Jose, CA, USA) with an associated Xcalibur (v. 1.2) data system. The standard Finnigan electrospray interface was operated in the negative and positive ion modes. Operating conditions for the mass measurements were: capillary temperature, 200°C; capillary voltage, (±) 20.0 V; ion spray voltage, (±) 4.5 kV; sheath gas flow rate, 20 arbitrary units.

Chromatography was performed on a Waters 1100 series HPLC system (Agilent Technologies, Inc., CA, USA) equipped with a quaternary pump, an autosampler, a thermostatted column compartment, and a multiwavelength diode array detector. Data were acquired and processed using HP Chem Station for LC software from Hawlet-Packard. The column used was a Waters Symmetry® C18 Column (5 µm, 4.6 × 150 mm). The flow rate was maintained at 0.8 mL/min and the injection volume was 20 µL. The mobile phase was prepared daily and filtered through a 0.45 µm membrane filter.

II. *Commercial Tablet Formulation*

For the method development, a commercial tablet formulation (Croxilex®-BID 1,000 mg coated tablets from I. E. Ulagay, Turkey, Batch no. 401006) containing 875 mg of AMX, 125 mg of KLV was used. All the proposed methods were applied to this pharmaceutical preparation.

AMX, KLV, ASP and CLN were kindly donated by Turkish Pharmaceutical Industrial Firms.

III. *Standard Solutions*

Stock solutions of AMX (35 mg/100 mL), KLV (25 mg/100 mL), and ASP (30 mg/100 mL) and CLN (30 mg/100 mL) were prepared in water. For each set of experiment, fresh solutions were used to avoid the instability problem. A standard series of the solutions containing 21-49 µg/mL of AMX and 3-7 µg/mL of KLV were prepared from the stock solutions. For three methods, same stock solutions were used. For ESI-MS studies 30 µg/mL of IS was included in each solution. All the calibration equations were obtained by three different assays and the average was taken as the final results.

IV. *Tablet Analysis*

For the preparation of tablet solution, 10 tablets were weighted and powdered in a mortar. From the average of those 10 tablets, one tablet amount was weighted and dissolved in 100-mL water in a 100-mL calibrated volumetric flask. The solutions were then filtered through 0.45 µm disposable membrane filter. The final solution was diluted to the working concentration for the experiments.

RESULTS AND DISCUSSION

Absorption spectra of both AMX and KLV in water are shown in Figure 1. The absorption spectrum of compounds reflects the dramatic concentration difference between two active ingredients and indicates some limitations for the spectrophotometric resolution of overlapped spectra of both compounds. This mixture is a perfect model to demonstrate the advantage of methods for the quantitation of both active ingredients of tablets.

First derivative method using zero crossing point[7-12] provides the resolution of active compounds in a mixture. Zero crossing method has some disadvantages for resolving overlapped spectra of components in a mixture. There are risk of small drifts of the working wavelengths and circumstance that the working wavelengths generally do not fall in correspondence of peaks of the derivative spectrum. This could be even more dangerous when the slope of the spectrum is very high with consequent loss of accuracy and precision, and the working wavelength is in the proximity of the base of the spectrum, which causes poor sensitivity[19].

Corresponding first derivative spectra of AMX and KLV for the calibration step are shown in Figure 2. While AMX provides satisfactory signal intensity for the appropriate calibration, KLV does not show enough clarity for a better calibration. First derivative also increases the noise level of spectrum and makes it harder to read

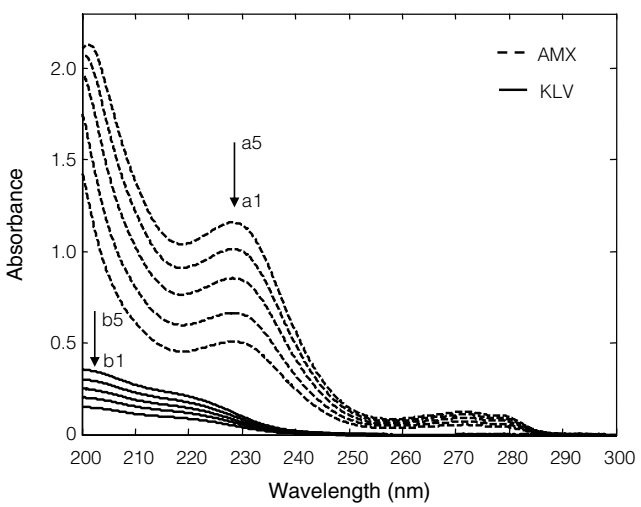

Figure 1. Absorption spectra of AMX (---) and KLV (—) in water. a1 to a5 represents concentration of AMX from 21 to 49 µg/mL. b1 to b5 represents concentration of KLV from 3 to 7 µg/mL.

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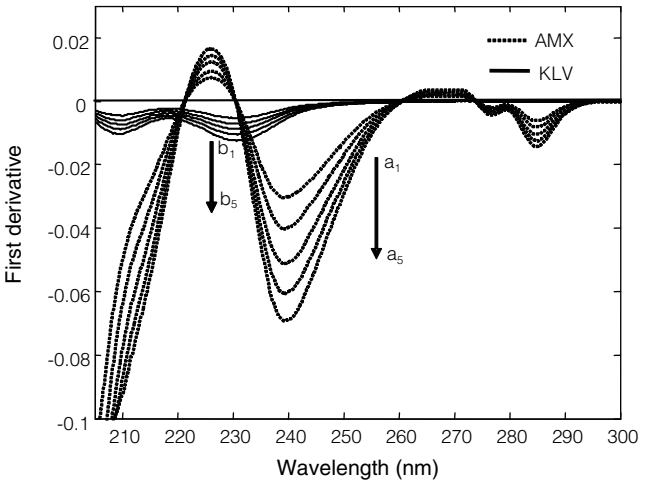

Figure 2. First derivative spectra of AMX (---) and KLV (—) in water. a1 to a5 represents concentration of AMX from 21 to 49 μg/mL. b1 to b5 represents concentration of KLV from 3 to 7 μg/mL.

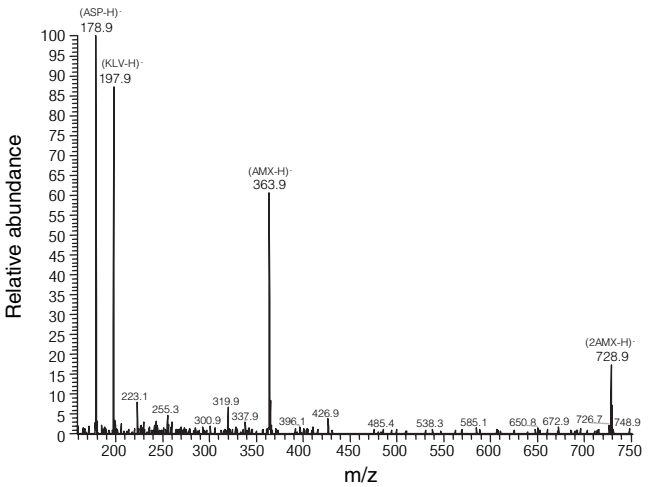

Figure 3. Negative ion ESI-MS spectra of tablet formulation of AMX 35 μg/mL, KLV 5 μg/mL and IS 30 μg/mL in water. MS conditions: negative ESI mode; heated capillary temperature, 200°C; capillary voltage, -4.5 kV; sheath gas flow rate, 20 arbitrary units.

the signal intensity at certain zero crossing points.

In derivative spectra, one of the main parameter that affects the signal to noise (S/N) ratio and peak shape is the Δλ value. This parameter needs to be optimized to give a well resolved large peak, i.e. to give good selectivity and high sensitivity in the determination. At higher Δλ, the S/N ratio becomes better, so that the fluctuation in a derivative spectrum decreases. However, if Δλ is too large, the spectral intensity of second derivative deteriorates. Various Δλ were tested and Δλ = 5 nm was chosen as the optimum in order to give an adequate S/N ratio.

Derivative spectrophotometry consists of calculating and plotting the mathematical derivatives of a spectrum. Thus, it offers a convenient solution to a number of analytical problems, such as resolution of multicomponent systems, reduced sample turbidity and enhancement of spectral details. The theoretical aspects of derivative spectrophotometry can be found elsewhere[7-12]. The significant resolution enhancement of derivative UV spectrophotometry has been applied advantageously to the determination of drugs.

It is well known that higher orders of derivative yield sharper peak amplitudes, but also yield lower derivative absorbance units of the bandwidth for a given curve[19]. The best selection for the derivative order is achieved where a greater reduction of the broad interfering exist. First derivative method was selected in our case because of low absorbance of KLV.

Negative ion ESI-MS spectra of subjected compounds with existence of internal standard are shown in Figure 3. Spectra were taken without any prior separation step. All the peaks in the spectra were determined and their peak heights were used for the quantitative purpose.

Acquisition parameters were determined by direct injection of a 5 μL/min solution of mixture of three compounds into the mass spectrometry. Variable mass spectrometric conditions, such as ESI probe temperature, capillary voltage and cone voltage, were investigated. ESI probe temperature was set at the minimal acceptable value (200°C). Capillary voltage was kept at -4.5 kV for the detection of AMX and KLV in the presence of IS. In the case of AMX, the deprotonated species, $[AMX-H]^-$, at m/z 363.9 was given after ionization in the electrospray source. The negative ion ESI spectrum of KLV has a deprotonated peak $[KLV-H]^-$, at m/z 197.9. The deprotonated molecular ion $[M-H]^-$ of IS at m/z 178.9 was recorded for quantitative purpose. In this study, negative ion ESI-MS spectra of tablet solution of these two compounds were shown to demonstrate that there is no interference from the tablet excipients. The peak at 728.9 amu corresponds to the dimer of AMX, $[2AMX-H^+]^-$ in solutiuon and its peak intensity increases as the concentration increases. This situation also gives another alternative to quantitate AMX in solution.

We also tried to develop another ESI-MS method by running the instrument in positive ion mode. Basically KLV by itself generated a lot of potassium and sodium complexes in positive ion mode. In the presence of other potassium grabber compounds like AMX those potassium complexes disappeared in KLV ESI-MS spectra profile.

In the positive ion mode, the potassium complex of AMX can be utilized to quantitate the amount of KLV. Because all the potassium was fromKLV thus the peak intensity of $[AMX+ K]^+$ can be used for quantitation. The spectrum of positive ion mode of tablet content is shown is shown in Figure 4. KLV spectrum of positive ion ESI-MS in water is shown in the insert. In positive ion mode CLN was chosen as an internal standard, because it does not form any potassium or sodium complexes and makes the quantitation possible in positive ion mode. Although CLN produces very good positive ion signals, it does not give any negative ion spectra. So we used

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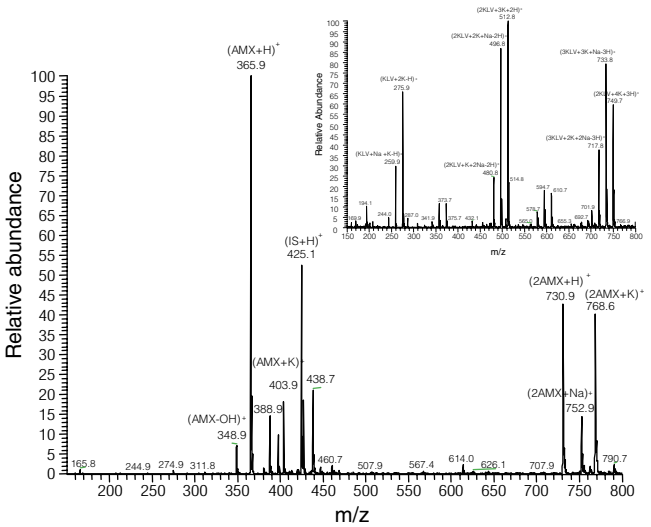

Figure 4. Positive ion ESI-MS spectra of tablet formulation of AMX 35 μg/mL, KLV 5 μg/mL and IS 15 μg/mL in water. The inset shows the positive ion spectra of KLV in water. MS conditions: positive ESI mode; heated capillary temperature, 200°C; capillary voltage, 4.5 kV; sheath gas flow rate, 20 arbitrary units.

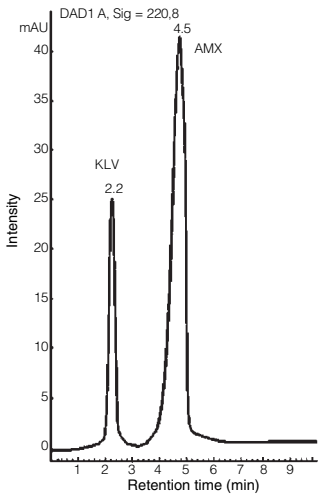

Figure 5. HPLC chromatogram of AMX and KLV in water. Chromatogram was taken at the highest concentrations (7 μg/mL for KLV and 49 μg/mL for AMX) of both drug.

ASP instead for the negative ion studies. The spectra also explained why the negative ion mode was chosen as the main method for the quantitative ESI-MS studies. The reason is that KLV peak lacks in positive ion spectra and the negative ion mode basically provides simple MS spectra for quantitative purpose.

In Figure 5, chromatograms of both compounds were shown. There is not any interfering peak in the chromatogram. The retention time of AMX and KLV was 2.2 and 4.5 min, respectively. The chromatographic running time of 6 min was appropriate for routine sample analysis. No internal standard was used in this chromatographic study. For the normalization of each chromatographic run, the peak area of AMX with the highest concentration was used.

I. *First Derivative Method*

First derivative of the spectra were calculated with the interval Δλ of 5 nm and resulting spectra were shown in Figure 2. For the best calibration, various Δλ values were tried and Δλ = 5 was found most suitable for the calculations. After derivation of the absorption spectra of compounds, zero crossing points were determined to establish two different calibration equations. For each compound, a graph was plotted by concentration versus absorbance and yields a straight line that can be used for the calculation of concentration calculation in synthetic mixtures or tablets. The same method can be applied to determine the concentration of other compound. The amounts of AMX and KLV in the binary mixture were found to be proportional to the signals and statistical parameters of their calibration equations are summarized in Table 1.

II. *ESI-MS Method*

Negative and positive ion ESI-MS spectra of two drugs and IS are shown in Figures 3 and 4, respectively. In negative ion spectrum, ASP was used as an internal standard and it is possible to see all the compounds and additional peaks. AMX also gives protonated dimer form in the solution, so we included all the related peaks for same compounds by adding up their intensities for the best calibration. In positive ion ESI-MS CLN was used as an internal standard and additional peaks were produced, so we follow the same procedure for calculations. Basically, various peaks were produced depending on heated capillary temperature, capillary voltage, skimmer voltages and other parameters in ESI source. The source conditions were optimized to suppress the adduct formation and fragmentation of compounds. Calibration standards of the AMX, KLV and IS were used to carry the calibration procedure. Ratios of the peak area signal of AMX or KLV to that of the IS were calculated. Linear relationships between the ratios of the peak area of the analytes to that of the IS and the corresponding concentrations were observed and illustrated by the equations in Table 1. In all the cases, back-calculated concentrations in the calibration curves were within 5% of the nominal values and the linear model satisfactorily describes the relationship between concentration and response.

III. *HPLC Method*

The given chromatogram in Figure 5 corresponds to the concentration of 49 μg/mL for AMX and 7 μg/mL for KLV. The detector responses were measured in terms of peak area. Chromatographic separation was carried out at the ambient temperature on a Waters Symmetry® C18 Column and the mobile phase consisted of 0.1 M

Table 1. Statistical results of calibration graphs obtained by three different methods

Method	Drug	λ (μv)	Regression equation	r[a]	SE (m)[b]	SE (n)[c]	SE (r)[d]
D[e]	AMX	284.4	A[f]= -2.91E-04C[g]$_{AMX}$-1.20E-05	0.9997	6.15E-06	2.24E-04	1.36E-04
	KLV	230.4	A= 8.88E-04C_{KLV} - 1.76E-02	0.9991	2.12E-06	2.21E-04	1.34E-04
ESI-MS negative	AMX	-	A= 3.56E-02C_{AMX} - 2.95E-01	0.9957	1.40E-03	4.91E-02	2.99E-02
	KLV	-	A= 7.67E-02C_{KLV} - 2.72E-01	0.9919	4.00E-03	4.15E-02	2.52E-02
ESI-MS positive	AMX	-	A= 6.67E-02C_{AMX} - 2.03E-01	0.9965	2.30E-03	8.29E-02	5.04E-02
	KLV	-	A= 1.95E-01C_{KLV} - 8.81E-01	0.9981	4.90E-03	5.11E-02	3.11E-02
HPLC	AMX	220	A= 2.46E-02C_{AMX} - 1.93E-01	0.9991	5.91E-04	2.15E-02	1.31E-02
	KLV	220	A= 2.06E-02C_{KLV} - 6.40E-03	0.9973	8.75E-04	9.10E-03	5.50E-03

[a]r: regression coefficient.
[b]SE (m): standard deviation of slope.
[c]SE (n): standard deviation of intercept.
[d]SE (r): standard deviation of linear regression.
[e]D: first derivative.
[f]A: amplitudes at selected wavelength for AMX and KLV.
[g]C: concentration (μg/mL).

phosphate buffer (pH = 8) and acetonitrile (v/v, 60:40). No internal standard was used during the studies. For the calibrations, all the peaks were normalized using the total peak area of compounds versus the peak area of individual drug in each chromatogram.

All the experimental and instrumental parameters were optimized to obtain the best S/N ratio for the subjected methods. The signal intensity of the compounds is important for the derivative calculations due to the intensity decrease after the derivation process. For ESI-MS case, signal intensity will not be a problem due to the low detection limit of mass spectrometry. In this method, the important factors for a robust method are the preparation of solutions and ESI source parameters.

IV. *Method Validation*

Five-point calibration curves were constructed for both drugs over the concentration range of 21-49 μg/mL for AMX and of 3-7 μg/mL for KLV. This concentration range was selected based on the anticipated drug concentration in absorption studies and used for spectrophotometric method development. In ESI-MS and HPLC studies, the plot of normalized peak areas of drugs versus concentrations was found to be linear within this concentration range. The similarity observed between the regression equations of individual pure drug and mixture solutions suggested no interferences in the estimation of one drug in the presence of the other. The regression equations are calculated from the calibration graphs, along with the standard deviations of the slope and the intercept. The linearity of calibration graphs and conformity of the absorption measurements to Beer's law were proved by the high values of the correlation coefficients (r) of the regression equations. In Table 1, all the obtained calibration equations and error values are shown.

The accuracy and selectivity of the proposed methods were verified by means of recovery assays for AMX and KLV in pure form and for the synthetic mixtures of both drugs and the excipients present in the tablets. Synthetic mixtures were prepared by diluting the stock solutions used for calibration. The percent recoveries of methods were found to be around 100 ± 5% (Table 2).

The interference of excipients in the pharmaceutical tablet was examined in detail by three methods. Standard addition technique was applied to commercial tablets containing these two compounds. In the standard addition method, standard deviations, standard errors and relative standard deviations for the proposed methods for six replicate were calculated as shown in Table 3. A good precision and accuracy was observed for these methods. Consequently the excipients in tablets do not interfere with the analysis of two compounds. Those results also indicate that there is not much suppression effect from the excipients of tablets.

The detection limits (LOD) and the quantification limits (LOQ) were calculated from the calibration data and shown in Table 4. LOD and LOQ determine the sensitivity of the method. LOD is the lowest concentration of the analyte detected by the method; whereas, LOQ is the minimum quantifiable concentration. The LOD was found to be at the levels of 0.87 and 0.47 μg/mL for AMX and KLV respectively. The LOQ were found to be 2.01 and 0.99 μg/mL for AMX and KLV, respectively. The selectivity of the method was determined by testing pharmaceutical preparation. Both the detection, and the quantification limits were in the acceptable range. Detailed information about LOD and LOQ for AMX and KLV for four methods is shown in Table 4.

The proposed methods were evaluated in the assay of commercial tablets. Six replicate determinations were

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Table 2. Recoveries obtained for the determination of AMX and KLV in different synthetic mixtures by proposed calibration techniques

Added		Found								% Recovery							
		D		ESI-MS negative		ESI-MS positive		HPLC		D		ESI-MS negative		ESI-MS positive		HPLC	
AMX	KLV	AMX	KLV	AMX	KLV	AMX	KLV	AMX	KLV	AMX	KLV	AMX	KLV	AMX	KLV	AMX	KLV
49	5	47.66	4.76	49.84	4.90	49.21	5.17	48.49	4.85	97.3	95.2	101.7	97.9	104.5	102.8	99.0	96.9
42	5	42.19	4.74	42.63	5.09	42.22	5.06	41.61	4.85	100.5	94.8	101.5	101.8	101.0	107.4	99.1	97.0
35	5	34.98	5.17	35.72	5.05	35.43	5.05	34.67	4.79	99.9	103.4	102.1	101.0	102.2	97.7	99.1	95.8
28	5	28.17	5.00	27.59	5.03	27.60	5.01	28.00	4.81	100.6	100.0	98.5	100.6	97.9	100.6	100.0	96.1
21	5	20.83	4.84	20.86	5.15	21.23	4.98	20.99	4.88	99.2	96.7	99.3	102.9	100.1	106.4	99.9	97.6
35	7	34.64	6.69	35.75	6.93	35.57	7.16	34.80	6.74	99.0	95.5	102.1	99.0	103.1	103.7	99.4	96.2
35	6	35.18	5.92	35.77	6.01	35.85	5.89	35.16	5.90	100.5	98.7	102.2	100.1	101.7	101.4	100.5	98.3
35	5	34.77	4.85	35.56	5.00	35.36	5.13	34.85	4.79	99.3	96.9	101.6	100.0	102.3	103.2	99.6	95.7
35	4	34.89	4.23	35.22	4.12	35.43	4.09	34.68	4.00	99.7	105.6	100.6	103.0	100.9	97.5	99.1	99.9
35	3	35.35	2.95	35.22	3.11	35.29	3.09	34.41	3.03	101.0	98.1	100.6	103.5	99.6	105.5	98.3	100.9
49	4	48.25	4.18	49.65	4.00	49.28	4.15	47.95	3.92	98.5	104.4	101.3	99.9	102.8	95.7	97.9	97.8
42	5	43.48	5.23	42.91	5.09	42.49	5.04	41.81	4.80	103.5	104.6	102.2	101.8	98.7	97.3	99.5	95.9
28	6	27.64	6.17	27.58	6.11	27.81	5.89	28.21	5.96	98.7	102.8	98.5	101.8	99.8	99.0	100.7	99.2
21	7	20.79	6.83	20.99	7.00	21.09	7.15	21.09	6.87	99.0	97.5	99.9	100.0	100.9	102.6	100.4	98.1
									Mean	99.8	99.6	100.9	101.0	101.1	101.5	99.5	97.5
									SD[a]	1.46	3.84	1.33	1.61	1.81	3.66	0.80	1.62
									RSD[b]	1.47	3.85	1.32	1.59	1.79	3.60	0.81	1.66

[a]SD: standard deviation.
[b]RSD: relative standard deviation.

Table 3. Results of standard addition method applied to commercial tablet preparation by the proposed calibration techniques

	D		ESI-MS negative		ESI-MS positive		HPLC	
	AMX	KLV	AMX	KLV	AMX	KLV	AMX	KLV
Mean	34.9	4.83	34.85	4.91	35.21	5.04	34.65	4.83
SD	1.22	0.13	0.92	0.06	1.52	1.59	0.09	0.11
RSD	3.5	1.31	2.63	0.64	1.51	1.58	0.27	1.16
SE	0.71	0.07	0.53	0.04	0.62	0.65	0.05	0.06
CL (0.05)	2.81	0.29	2.11	0.14	1.22	1.27	0.22	0.26

made. As shown in Table 4 appropriate results were obtained for the recovery of both drugs and the data were in good agreement with the label claim. To validate the derivative spectrophotometric method, the data were compared with those obtained by the HPLC method. Results of the two commercial tablets are also given in Table 4.

One way ANOVA (analysis of variance) was applied to the results of tablet for three methods. Test results were tabulated in Table 4. The calculated F values were lower than the tabulated F-value indicating that there is not much difference between the methods.

All the methods presented in this paper, enable the quantification of mixtures of AMX and KLV with good accuracy and precision. The results show that the application of ESI-MS method brings considerable advantage for the quantification of drugs with low absorbance. The advantage of the method basically comes from its simplicity. It is also a fast and economical method. It has, however, some limitation, e.g. ion suppressions, complex formations and availability of proper internal standards for quantitation.

The results displayed give full evidence for the usefulness, reliability and repeatability of the methods, which have been also validated to be precise, sensitive and accurate. It is obvious that HPLC provide better results than other two, but it generally needs extra time and cost for the method development and sometimes it may fail to resolve those components in a mixture. The other two methods did not cost the elaboration of treatment procedures. Hence they are generally fast and

Table 4. Results obtained in the pharmaceutical dosage forms by the proposed calibration methods

	D		ESI-MS negative		ESI-MS positive		HPLC	
	AMX	KLV	AMX	KLV	AMX	KLV	AMX	KLV
Mean	35.05	4.81	34.7	4.96	35.2	5.05	34.2	4.82
SD	0.99	0.07	0.77	0.13	1.99	0.63	0.08	0.11
RSD	2.82	0.73	2.21	1.28	1.98	0.62	0.22	1.19
SE[a]	0.57	0.04	0.44	0.07	0.81	0.26	0.04	0.07
CL[b] (0.05)	2.26	0.16	1.76	0.29	1.59	0.5	0.17	0.26
ANOVA	0.5344	0.9217	0.5344	0.9217	0.5344	0.9217	0.5344	0.9217
$F_{theoretical}$	3.6823	3.6823	3.6823	3.6823	3.6823	3.6823	3.6823	3.6823
LOD	0.8752	0.6258	0.7713	0.3255	0.6534	0.5674	0.7821	0.475
LOQ	2.0056	1.5095	1.8939	0.995	1.9546	1.2342	2.0772	1.1389

Lable claim (mg): 875 mg AMX, 125 mg KLV per tablet. The mean values show 25 times diluted concentration values.
Results obtained are average of 6 replicate for each method.
[a]SE: standard error.
[b]CL: confidential limit.

economical in comparison to the time-consuming chromatographic techniques.

CONCLUSIONS

The purpose of this work was to compare the proposed spectrophotometric and ESI-MS methods. It is shown that ESI-MS method can be used for the pharmaceutical field by giving more selection for the calibrations and the results has been validated with the HPLC results. Although the relative absorption of AMX and KLV is quite different in UV spectrum, we have shown that the first derivative method can be used for quantitation. ESI-MS provides better and distinct peaks without being affected by the dramatic concentration difference between two active compounds. It is also shown that these two compounds can be easily quantitated by both ESI-MS and the first derivative methods without any separation and excipient effect. The developed HPLC method also provides the tablet analysis and dissolution studies in acceptable range.

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