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Determination of phenacetin and salophen analgetics in solid binary mixtures with caffeine by infrared linear dichroic and Raman spectroscopy

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Abstract

Quantitative infrared (IR) and Raman spectroscopic approach for determination of phenacetin (*Phen*) and salophen (*Salo*) in binary solid mixtures with caffeine: phenacetin/caffeine (*System 1*) and salophen/caffeine (*System 2*) is presented. Absorbance ratios of 746 cm^{-1} or 721 cm^{-1} peaks (characteristic for each of determined compounds in the *Systems 1* and *2*) to 1509 cm^{-1} and 1616 cm^{-1} (attributed to *Phen* and *Salo*, respectively) were used. The IR spectroscopy gives confidence of 98.9% (*System 1*) and 98.3% (*System 2*), while the Raman spectroscopic data are with slightly higher confidence of 99.1% for both systems. The limits of detection for the compounds studied were 0.013 and 0.012 mole fraction for IR and Raman methods, respectively. Solid-state linear dichroic infrared (IR-LD) spectral analysis of solid mixtures was carried out with a view to obtaining experimental IR spectroscopic assignment of the characteristic IR bands of both determined compounds. The orientation technique as a nematic liquid crystal suspension was used, combined with the so-called reducing-difference procedure for polarized spectra interpretation. The possibility for obtaining supramolecular stereo structural information for *Phen* and *Salo* by comparing spectroscopic and crystallographic data has also been shown. An independent high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) analysis was performed for comparison and validation of vibrational spectroscopy data. Applications to 10 tablets of commercial products APC and Sedalgin are given.

Keywords: Phenacetin; Salophen; Caffeine; IR-LD and Raman spectroscopy; Solid sampling

1. Introduction

The considerable commercial interest of pharmaceutical industry in phenacetin (*Phen*), *N*-(4-ethoxyphenyl)ethanamide or *p*-acetophenetidine, and salophen (*Salo*), acetaminosalol or 4-acetamidophenyl salicylate (Fig. 1) is due to their wide usage as antipyretic [1], analgetic [2–6] and anti-inflammatory agents [7,8]. The bulk of clinical evidence linking analgetic abuse with chronic renal disease is overwhelming and analgetic nephropathy continues to be an important problem. The characteristic lesion is renal papillary necrosis with secondary cortical damage, leading to progressive renal failure. The development of uroepithelial tumors represents a further serious long-term complication. The large majority of reports of analgetic nephropathy have involved analgetic mixtures containing phenacetin with aspirin or pyrazolones such as antipyrine and amidopyrine, together with caffeine and sometimes codeine and barbiturates. This is because analgetic abusers prefer combination to single drug products, and until the use of phenacetin was restricted it was present in most popular analgetic combinations [9]. The role of caffeine as a promoter of analgetic-associated nephropathy has been documented [10]. The interactive effects of caffeine and phenacetin on the locomotor activity involve changes in absorption and metabolism as well as effects possibly exerted at the CNS level. Phenacetin initially retarded the absorption of caffeine when coadministered by gavage but not when caffeine was given intraperitoneally and phenacetin orally [11]. For these reasons there is an ever-growing interest for fast,

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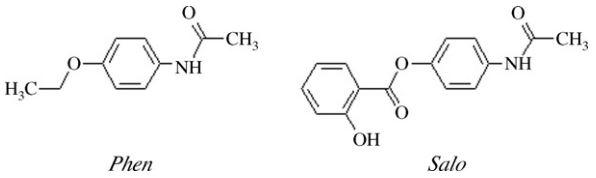

Fig. 1. Chemical formula of phenacetin (*Phen*) and salophen (*Salo*).

simple and reliable analytical methods for the determination of these pharmaceutical products in mixtures. Several instrumental techniques have been applied in this field. Quantitative NMR assay for aspirin, phenacetin and caffeine mixtures with 1,3,5-trioxane as internal standard has been described [12]. Dou at al. [13] analysed aminopyrine/phenacetin tablets by near IR spectroscopy by applying artificial neural networks combined with principal components analysis. Analgetic mixtures of phenazone, phenacetin and caffeine in the presence of some of their degradation products have been quantitatively characterized [1]. Caffeine in aspirin/phenacetin/caffeine tablets has been quantified by semi-automated UV spectroscopy [14]. Simultaneous quantification of acetaminophen, aspirin, caffeine, codeine phosphate, phenacetin and salicylamide by high-performance liquid chromatography has been described [15], while acetaminophen, caffeine and chlorphenamine maleate in paracetamol and chlorphenamine maleate granules have been simultaneously determined [16]. All these methods as well as powder X-ray diffraction, are powerful and routine techniques for the identification of mixtures. The latter X-ray technique provides best selectivity. Most of these techniques are relatively expensive and require extensive preliminary sample treatment.

The aim of this work was to elaborate a method for quantification of phenacetin (*Phen*) and salophen (*Salo*) (Fig. 1) in solid binary mixtures based on IR and Raman spectroscopy, which is relatively cheap, fast and easy for technical operation, interpretation of data and does not require sample dissolution. The linear-polarized infrared (IR-LD) spectral analysis of oriented solids *Phen* and *Salo* as a liquid crystal suspension was applied for experimental IR band assignment and selection of appropriate bands for quantitative determination. This method gives additional supramolecular solid-state structural information at room temperature and atmospheric pressure. It also avoids the phase transition [17,18] and guarantees the study of different forms without polymorphs transitions. This approach has been applied recently for matrix compound caffeine and for studying the polymorphs of paracetamol and aspirin [19].

2. Experimental part

2.1. Materials and methods

Phenacetin and salophen (Sigma–Aldrich) and caffeine (Merck) were used.

The tablets of pharmaceutical commercial products APC and Sedalgin tablets were purchased from RITE AID (USA) and Sopharma Ltd. (Bulgaria).

The IR spectra were measured on a Thermo Nicolet FTIR spectrometer 7000 (4000–400 cm^{-1}, 0.5 cm^{-1} resolution, 150 scans) equipped with a Perkin-Elmer wire-grid polarizer. Non-polarized solid-state IR spectra were recorded using the KBr disk technique at ambient conditions ($T = 298$ K, $P = 1$ atm). The oriented samples were prepared as a suspension in a nematic liquid crystal (MLC 6815, Merck) with the presence of an isolated nitrile stretching IR band at about 2230 cm^{-1}, additionally serving as an orientation indicator. This approach has been presented recently by Ivanova et al. [20]. It has been validated for accuracy, precision and the influence of the liquid crystal medium on peak positions and integrated absorbances of the guest molecule bands [21,22]. The number of scans, the rubbing-out of KBr-pellets, the amount of studied compounds included in the liquid crystal medium and the ratios of Lorentzian to Gaussian peak functions in the curve fitting procedure on the spectroscopic signal at five different frequencies has been studied. It has been found that the procedure for the position (ν_i) and integrated absorbances (A_i) determination for each i-peak have been carried out by deconvolution and curve-fitting procedures at 1:1 ratio of Lorentzian to Gaussian peak functions, χ^2 factors within 0.00033–0.00023 (in our cases) and 3000 iterations. The means of two treatments were compared by Student's t-test, using Statistica 5.11 software. The experimental IR-spectral patterns have been acquired and processed by GRAMS/AI 7.01 IR spectroscopy (Thermo Galactic, USA) program package. The applicability of this approach for experimental IR spectroscopic band assignment and for obtaining stereo-structural information has been demonstrated in series of organic systems and coordination complexes as polymorphs [19], heterocyclic, Cu(II) complexes, codeine derivatives, peptides and their Au(III) complexes, hydrochlorides and hydrogensquarates [23,24].

Raman spectra in solid-state were recorded on Horiba Jobin-Yvon Raman spectrometer.

HPLC–MS/MS measurements were made using TSQ 7000 instrument (Thermo Electron Corporation) under the conditions presented in Table 1. Two mobile phase compositions were used: (A) 0.1% v/v aqueous HCOOH and (B) and 0.1% v/v HCOOH in CH_3CN.

Electrospray ionization (ESI) mass spectrometry: A triple quadruple mass spectrometer (TSQ 7000 Thermo Electron,

Table 1
Calibration points on the regression lines of IR and Raman spectroscopic methods for the determination of *Phen* and *Salo* in binary mixtures with caffeine (*Systems 1* and *2*)

Reciprocal mole fraction ($1/X_i$)	Peak ratios $A^t/A_{\nu 1}$			
	System 1		*System 2*	
	A_{746}/A_{1509}	A_{746}/A_{1509}	A_{721}/A_{1616}	A_{721}/A_{1616}
	IR	Raman	IR	Raman
1.11	0.055	0.092	0.325	0.032
1.25	0.135	0.097	0.380	0.040
1.42	0 140	0.109	0.398	0.050
1.67	0.170	0.113	0.400	0.080
2.00	0.180	0.250	0.415	0.090
2.50	0.210	0.300	0.440	0.150
3.33	0.300	0.400	0.487	0.250
5.00	0.410	0.630	0.550	0.450
10.00	0.781	1.224	0.783	0.948

Dreieich, Germany) equipped with an ESI 2 source was used and operated at the following conditions: capillary temperature 180 °C, sheath gas 60 psi, corona 4.5 μA and spray voltage 4.5 kV. Sample was dissolved in acetonitrile (1 mg ml^{-1}) and was injected in the ion source by an autosampler (Surveyor) with a flow of pure acetonitrile (0.2 ml min^{-1}). Data processing was performed by Excalibur 1.4 software.

Samples for HPLC–MS/MS was done using the procedure described for quantitative determination of caffeine in ACP tablets by thin liquid chromatography (TLC) and HPLC [25,26].

3. Results and discussion

The quantitative analysis requires an adequate identification of characteristic IR-spectral bands of *Phen* and *Salo* but the corresponding IR-spectroscopic patterns are complicated as a result of strong overlapping. In the case of *Salo* the presence of two different substituted phenyl fragments additionally complicates spectrum interpretation. The IR-LD characterization stated below cover these difficulties in significant level. Independently, in all cases the experimental IR curves are processed preliminary by deconvolution and curve fitting using a validated approach [21,22]. The analysis below has been aimed at selecting suitable for quantitative analysis bands. A single, characteristic for each of the compound studied, *Phen* and *Salo*, that does not overlap with the band of the matrix compound caffeine as well as a second peak typical for each of the compounds in the corresponding binary mixtures was sought for *Systems 1* and *2*.

The solid-state IR spectra in KBr-pellets and the non-polarized ones as nematic liquid crystal suspension of both compounds have shown identical spectral patterns, thus avoiding the effect of the orienting mesophase on the intensity and peak positions of the compound studied. The same refers to the matrix compound caffeine.

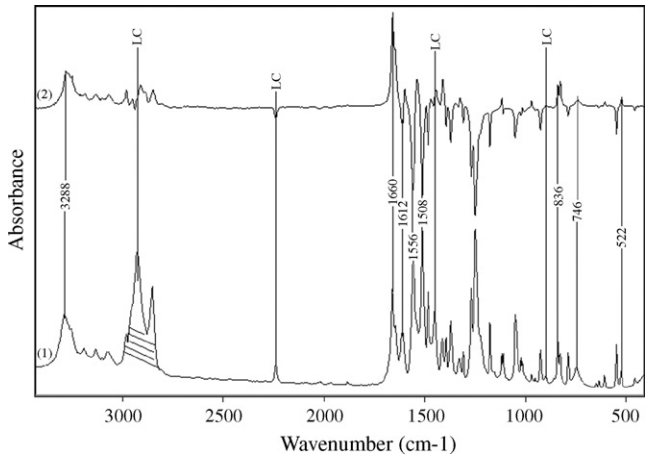

Fig. 2. Non-polarized (1) and difference IR-LD (2) spectra of *Phen* as nematic liquid crystal suspension.

The phenyl fragment vibrations assignment was performed in accordance with Wislon notation [27,28].

3.1. IR-LD spectral analysis

3.1.1. Phenacetin

The difference and non-polarized IR-LD spectra of *Phen* are shown in Fig. 2. Similar to analogous data for monoclinic paracetamol, the spectrum in Fig. 2(2) is characterized with positive peaks at 3289 cm^{-1} and 1660 cm^{-1}/1646 cm^{-1} corresponding to ν_{NH} and $\nu_{C=O}$ (Amide I) stretching modes of amide fragment. This assignment correlated with other published data [27]. The 1700–1450 cm^{-1} region showed a series of peaks oriented as follows at 1614 cm^{-1}, 1606 cm^{-1} (negatives, (−)) corresponding to **8a** (in plane), 1558 cm^{-1} (−) belonging to Amide II mode and 1509 cm^{-1} (−) for **19a** [33,34]. The 800–650 cm^{-1} region

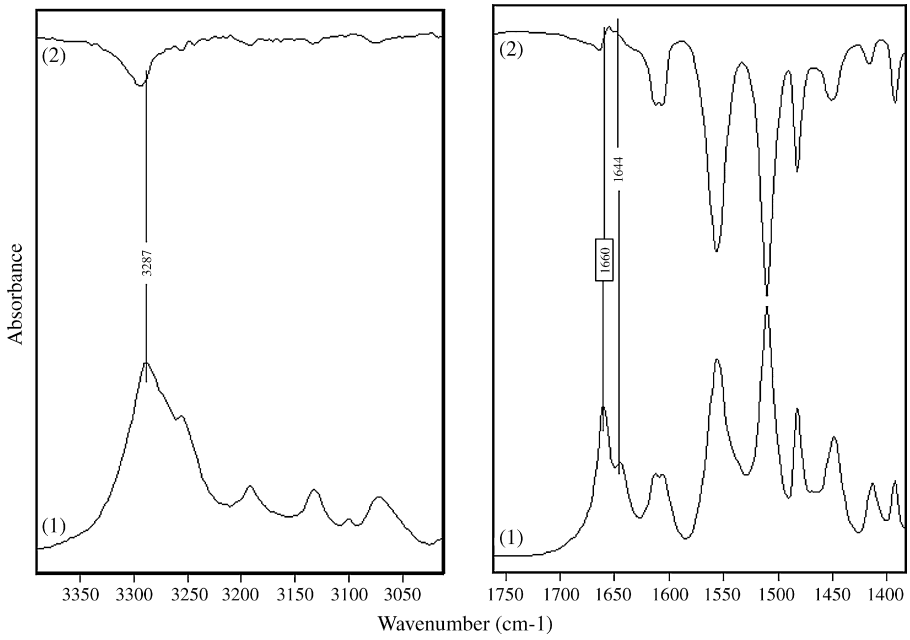

Fig. 3. Non-polarized (1) and reduced IR-LD (2) spectra of *Phen* after elimination of 1660 cm^{-1} peak.

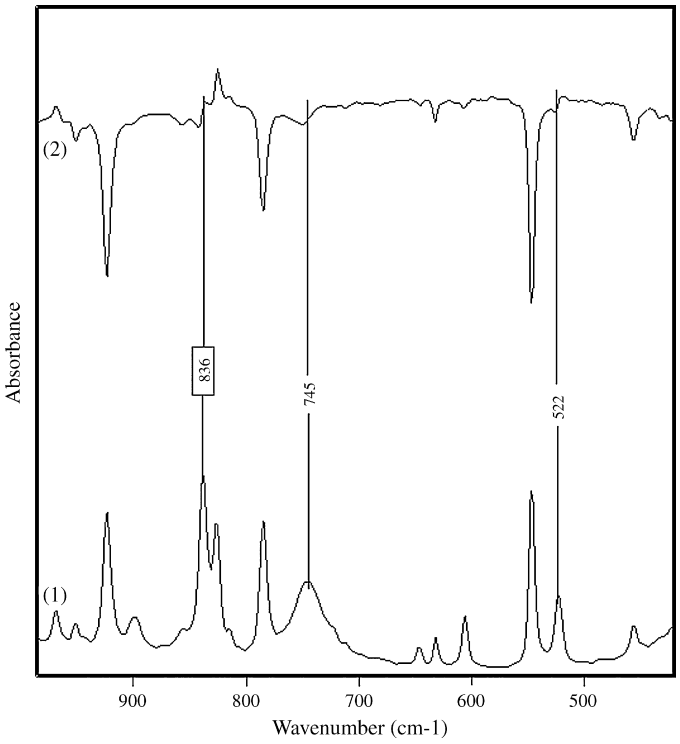

Fig. 4. 1000–400 cm^{-1} region of non-polarized (1) and reduced after elimination of 836 cm^{-1} peak (2) IR-LD spectra of *Phen*.

is characterized with 836 cm^{-1} (+), 825 cm^{-1} (+), 784 cm^{-1} (−) and 746 cm^{-1} (+) characteristic for aromatic systems. The values are typical for other *p*-substituted *N*-arylacetanilides [28]. Like the monoclinic paracetamol, the presence of cross-oriented *Phen* molecules in solid-state leads to simultaneous elimination of IR spectral maxima possessing to different symmetrical class in the corresponding polarized IR-LD spectra. Single crystal X-ray diffraction data of *Phen* [29–31] have shown crystalization in $P2_1/c$ cell setting and $Z=4$, where in the unit cell the four molecules are disposed mutually by pairs and the planes of benzene ring closing an angle of 88.5(4)°. Elimination of the 1660 cm^{-1} peak (Fig. 3(2)) provoked strong reduction of the 3289 cm^{-1} peak, thus confirming *trans*-configuration of amide fragment due to the dihedral angle O=C–NH of 178.8(2)° [31], causing the co-linearity of corresponding ν_{NH} and $\nu_{C=O}$ transition moments. In addition, disappearance of the 1644 cm^{-1} peak is also observed. The simultaneous elimination of both 1660 cm^{-1} and 1646 cm^{-1} bands assumed the crystal field splitting effect of Amide I maximum in solid *Phen*. The IR-LD spectral analysis of *Salo*, containing two types of substituted benzene rings required the assignment of **11**-γ_{CH} (out-of-plane mode) of *p*-substituted aromatic system. The elimination of 836 cm^{-1} maximum (Fig. 4(2)) leads to disappearance of the broad maximum at 746 cm^{-1} and the peak at 522 cm^{-1}, thus confirming the character of the first maximum as **11**-γ_{CH}.

3.1.2. Salophen

In contrast to *Phen*, the IR spectrum of *Salo* (compare Figs. 2(1) and 7) is characterized by high-frequency shift ν_{NH} and $\nu_{C=O}$ (Amide I) maxima to 3305 cm^{-1} and 1662 cm^{-1} due

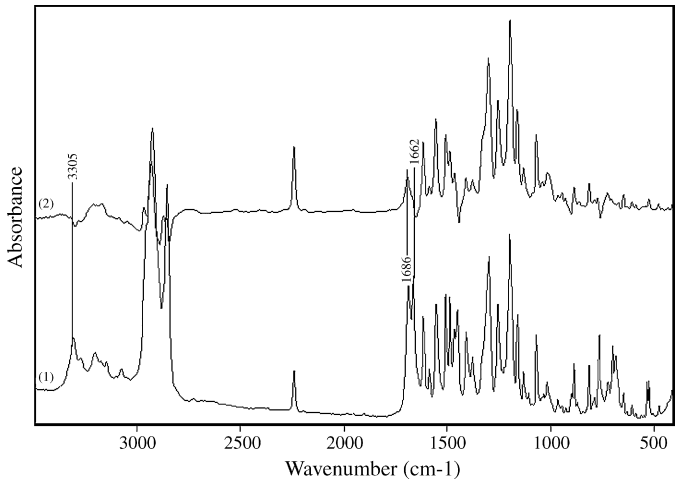

Fig. 5. Non-polarized (1) and difference IR-LD (2) spectra of *Salo* as nematic liquid crystal suspension.

to the presence of moderate NH···O=C intermolecular interaction, typical for other *p*-acetamidophenyl derivatives [32,33]. The NH-region contains the low frequency shift ν_{OH} absorption maximum at 3295 cm^{-1} of the salycylate OH group also included in OH···O=C intramolecular H-bond with neighboring salycylate O=C group. The Amide I stretching region shows an additional peak at 1685 cm^{-1}, characteristic of $\nu_{C=O}$ stretch of the salycylate structural fragment. In contrast to *Phen* the 850–650 cm^{-1} region of *Salo* indicated the low frequency shift **11**-γ_{CH} mode of *p*-substituted benzene ring from 836 cm^{-1} for *Phen* to 813 cm^{-1} for *Salo*. *Salo* has shown two additional peaks at 763 cm^{-1} and 698 cm^{-1}, presumably belonging to **11**-γ_{CH} and **4**-γ_{Ar} of the second salycylate aromatic ring. The assignment is carried out by means of IR-LD spectral analysis.

The difference IR-LD spectrum (Fig. 5(2)) of *Salo* is characterized with eliminated 3305 cm^{-1} and 1662 cm^{-1} peaks, thus indicating a collinear orientation of ν_{NH} and $\nu_{C=O}$ (Amide I) transition moment and *trans*-configuration of amide fragments like paracetamol [33] and phenacetin. The reduction of the 813 cm^{-1} peak and appearance of a positive one at 763 cm^{-1} is observed

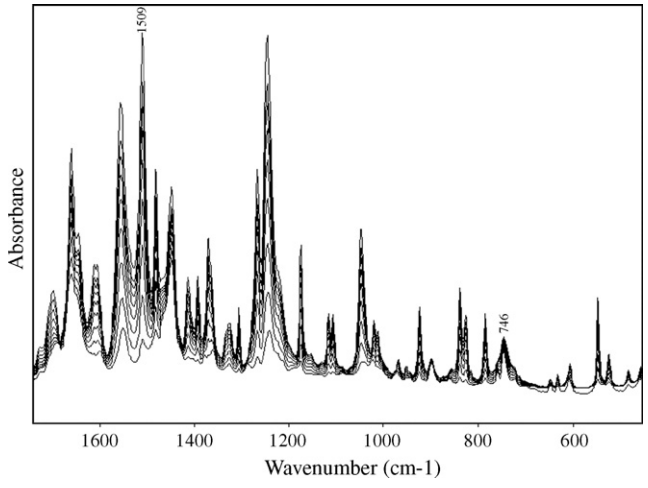

Fig. 6. IR-spectra of the solid-state mixtures of *System* 1 in different mole fraction of *Phen*.

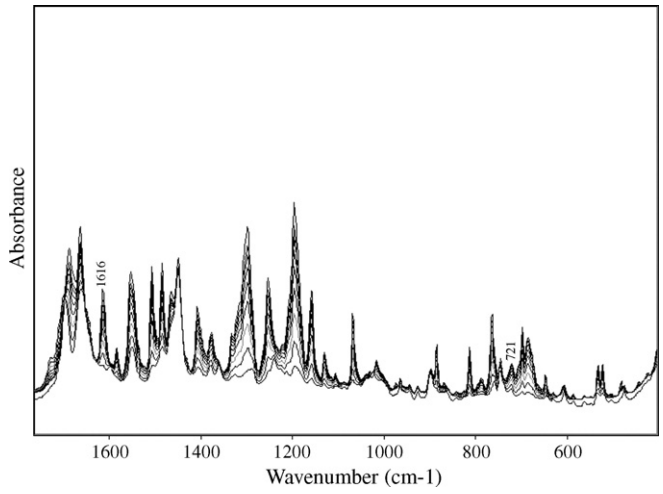

Fig. 7. IR-spectra of the solid-state mixtures of *System 2* in different mole fraction of *Salo*.

in the corresponding reduced IR-LD spectrum. The elimination of the **11**-γ_{CH} modes of both phenyl rings indicated a significant degree of deviation of the phenyl planes.

IR-LD spectroscopic characterization of both compound *Phen* and *Salo* as well as the analogous one of caffeine has shown that the band at 1509 cm^{-1} is typical only for *Phen* and that at 1616 cm^{-1} for *Salo*, respectively. The maximum at 746 cm^{-1} is typical for both *Phen* and caffeine (Fig. 6), while band at 721 cm^{-1} for *Salo* and caffeine (Fig. 7). Therefore, these maxima are appropriate for IR and Raman quantification. These bands are also observed in corresponding Raman spectra of compounds, but with different intensities.

3.2. Quantitative analysis

It is known that for a two-component mixture, the total absorbance A^t of the mixture at a given frequency is the sum of the absorbances of two component compounds, i and j, at the specified frequency: $A^t = A^i + A^j = a^i \times b \times c_i + a^j \times b \times c_j$. Molar absorbtivities a^i and a^j are determined from absorption measurements of mixtures containing known amounts of compounds i and j at two different frequencies, ν_1 and ν_2.

If the ratio of total absorbance of given band (A^t) to the absorbance of a second band (A^i), typical only for one component of mixture (*e.g. Phen* or *Salo*), then the following equations could be written as:

$$\frac{A^t_{\nu 1}}{A^i_{\nu 2}} = \frac{(a^i \times \nu_1 \times b \times X_i + a^j \times \nu_1 \times b \times X_j)}{(a^i \times \nu_1 \times b \times X_i)},$$

mole fractions of components are X_i and X_j and $X_i + X_j = 1$, then

$$\frac{A^t_{\nu 1}}{A^i_{\nu 2}} = \frac{(a^i \times \nu_1 \times b \times X_i + a^j \times \nu_1 \times b \times (1 - X_i))}{(a^i \times \nu_1 \times b \times X_i)}$$

$$\cdots$$

$$\frac{A^t_{\nu 1}}{A^t_{\nu 2}} = \left[\frac{(a^i \times \nu_1 - a^j \times \nu_1)}{a^i \times \nu_2}\right] + \left(\frac{a^j \times \nu_1}{a^i \times \nu_2}\right) \times \left(\frac{1}{X_i}\right)$$

The frequencies used are respectively:

for *System 1*: $\nu_1 = 746\ cm^{-1}$ and $\nu_2 = 1509\ cm^{-1}$
for *System 2*: $\nu_1 = 721\ cm^{-1}$ and $\nu_2 = 1616\ cm^{-1}$.

Similar equations are used for Raman measurements [34]. For adequate comparison of reliability of IR and Raman data, the same bands are utilized for quantitative analysis. In corresponding Raman spectra the character of the used bands is the same, but their intensity is different.

3.2.1. IR spectroscopy

The ratios of IR characteristic peaks of *Phen* (ν_2 1509 cm^{-1}) and *Salo* (ν_2 1616 cm^{-1}) with the peak at 746 cm^{-1} (ν_1) in *System 1* and 721 cm^{-1} (ν_1) in *System 2*, typical for both compounds in corresponding solid mixtures have been evaluated for quantification, using the above mathematical model. Repeated IR analyses of samples (three replicates) for each mole fraction and each system were applied (Fig. 8). The results of mean peak ratios are presented in Table 1. Linear regression analysis between contents and peak ratio data gave straight-line plots:

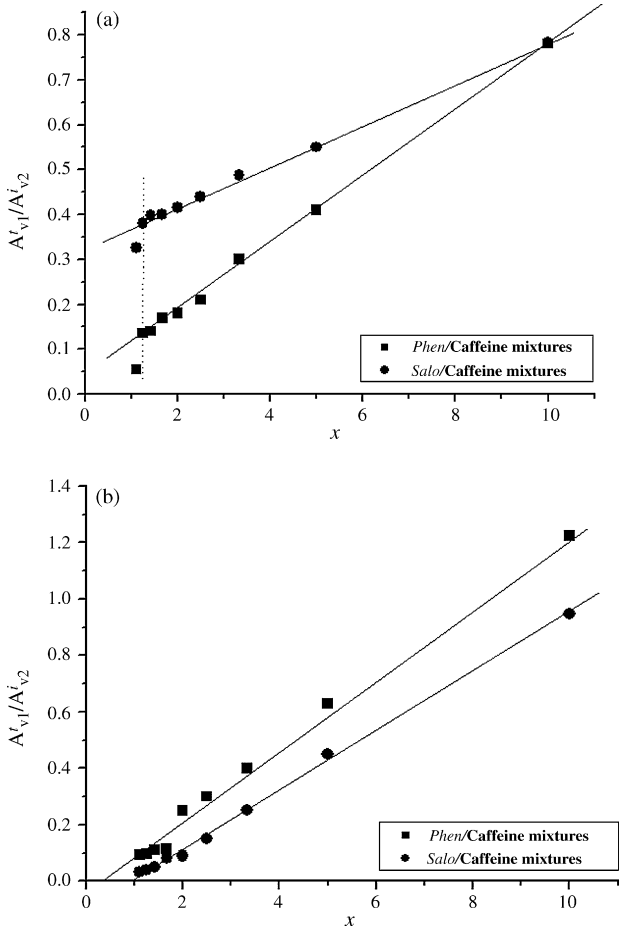

Fig. 8. IR-spectroscopic (a) and Raman (b) dependences of absorption peak ratios A_{746}/A_{1509} *vs.* x and A_{721}/A_{1616} ($x = 1/X_i$, where X_i is mole fraction of *Phen* or *Salo* in the mixtures with Caffeine).

Table 2
Regression analysis r and r^2 values

Compound	Method	r	r^2
Phen	IR	0.995	0.989
	Raman	0.996	0.993
Salo	IR	0.992	0.983
	Raman	0.999	0.998

System 1: $y = 0.02 \times (\pm 0.04) + 0.76 \times (\pm 0.05) \times x$, $x = 1/X_i$ with confidence intervals within 0.41–0.69 and 0.66–0.77 for slope and intercept, respectively;
System 2: $y = 0.31 \times (\pm 0.02) + 0.04 \times (\pm 0.05) \times x$, $x = 1/X_i$ with confidence intervals within 0.51–0.62 and 0.76–0.80 for slope and intercept, respectively.

The linear relationship is therefore:

System 1: $y = 0.024 + 0.076 \times x$
System 2: $y = 0.315 + 0.047 \times x$.

The corresponding correlation coefficients r and r^2 are given in Table 2. In all cases, $p < 0.0001$ values indicate significant correlation. The squared values r^2 (Table 2) give a confidence of 98.9% and 98.3% for both *Systems 1* and *2*.

3.2.2. Raman spectroscopy

Quantitative analysis of S*ystems 1* and *2* by means of Raman spectroscopy has also been performed with three replicates for each mole fraction. The results of means value for ratios of the bands area are presented in Table 1. Regression analysis of data gave a straight-line calibration (Fig. 8):

System 1: $y = -0.05 \times (\pm 0.01) + 0.12 \times (\pm 0.05) \times x$, where $x = 1/X_i$ with confidence intervals within 0.11–0.34 and 0.90–0.98 for slope and intercept, respectively;
System 2: $y = -0.09 \times (\pm 0.03) + 0.10 \times (\pm 0.05) \times x$, where $x = 1/X_i$ with confidence intervals within 0.81–0.90 and 0.55–0.70 for slope and intercept, respectively.

The linear relationship is:

System 1: $y = -0.050 + 0.129 \times x$
System 2: $y = -0.098 + 0.105 \times x$.

The r and r^2 coefficients, $p < 0.0001$ and values of r and r^2 (Table 2) are similar to those obtained by IR spectroscopy, with confidence of 99.3% and 99.8% for both *Systems 1* and *2*, which is a minor improvement *versus* the IR data.

The validity of these equations has been confirmed by means of the spectra of pure compounds from which the ratios a^i_{746}/a^i_{1509} and a^j_{721}/a^j_{1616} are 0.782 and 0.362, respectively. Taking into account that the absorption measured in the Raman spectrum depends on the intensity of the laser and on the factor K_ν including the frequency depending terms $A_\nu = I_o \times K_\nu \times X$ [34], the K^i_{746}/K^i_{1509} and K^j_{721}/K^j_{1616} ratios have been calculated. The values obtained: $0.78 \pm 1.3 \times 10^{-2}$ and $0.36 \pm 1.1 \times 10^{-2}$ are very close to experimental ones. The limit of detection (LOD) was estimated at 0.013 and 0.012 mole fraction for IR and Raman methods, respectively. Admittedly, the linear calibration range using IR and Raman method is confined to 0.8 and 0.9 mole fractions of determined compound, respectively, which is appropriate for concentration ranges of these binary mixtures in pharmaceutical analysis. The obtained better results of the Raman spectra, comparing with IR ones could be explained with the fact that in first case the observation of relatively small numbers and in many cases non-overlapped of bands allow a more precise determination of the integral absorbencies.

3.2.3. HPLC–MS-MS data

The correlations between the results for samples with different amounts of *Phen* and *Salo* in both systems obtained by spectroscopic and HPLC–MS/MS techniques demonstrate good agreement with correlation coefficients >0.9999 (Fig. 9).

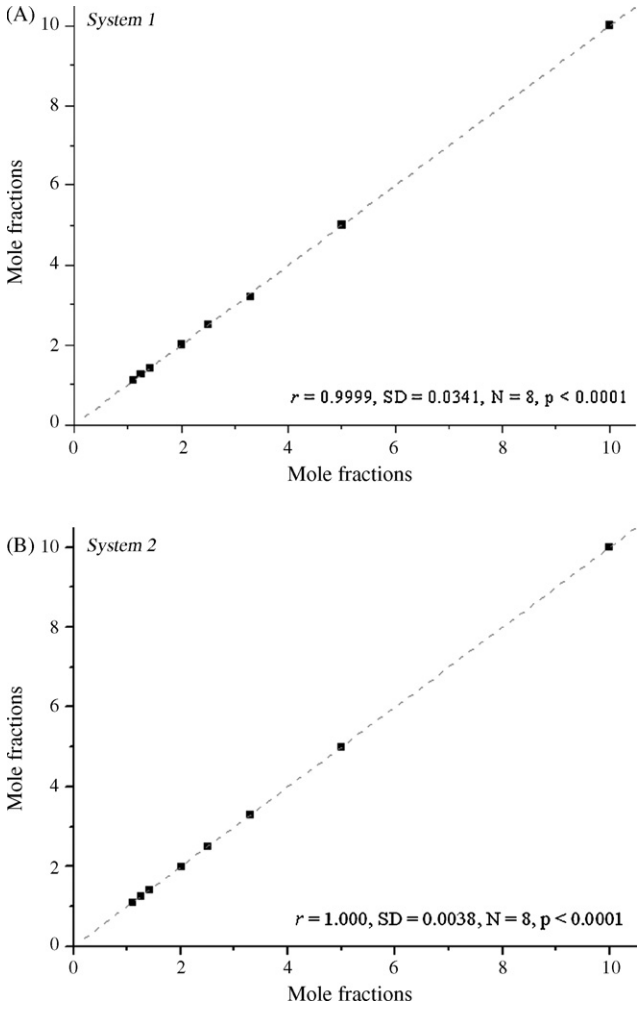

Fig. 9. Correlation between the spectroscopic and HPLC–MS-MS results (reciprocal value of each mole fraction) for *System 1* (A) and *System 2* (B).

3.2.4. Quantitative determination of Phen and Salo in commercial products ACT and Sedalgin tablets

The application of this mathematical model on real commercial products has been demonstrated on 10 different tablets of ACP and Sedalgin containing 200 mg of *Phen* in each product, which corresponds to 0.4 mole fraction or reciprocal value of 2.4. Three replicates were made for each sample. The IR measurements gave a standard deviation of 0.013 and 0.013 at $p = 0.0513$ and 0.0507 for both the systems, while the Raman technique gave slightly better p values of 0.067 and 0.077. The confidence of >99.98% was obtained using this vibrational model.

4. Conclusion

Quantitative determination of phenacetin and salophen in binary solid mixtures with caffeine, phenacetin/caffeine and salophen/caffeine was carried out using the possibilities of IR and Raman spectroscopy. The intensity ratio of 746 cm^{-1} peak and 721 cm^{-1} (characteristic for each of the determined compounds) to 1509 cm^{-1} and 1616 cm^{-1} (attributed only to *Phen* and *Salo*, respectively) was used. The choice was made using the possibilities of IR-LD spectroscopy of oriented solid samples as suspension in nematic liquid crystal as a tool for adequate experimental assignment of IR bands to corresponding vibrational modes in solid state. The IR spectroscopy gives confidence of 98.9% and 98.3% for both systems. Raman spectroscopic data have shown minor improvement to 99.1%. Linear calibration range using IR and Raman method is confined within 0.013–0.8 and 0.012–0.9 mole fraction for IR and Raman methods, respectively. These results together with recently reported applications to phenacetin and aspirin polymorphs clearly demonstrate the applicability of these spectroscopic tools for quantitative determination of pharmaceuticals constituents in solid-state binary mixtures. Independent HPLC–MS/MS analyses yield results in agreement with vibrational spectroscopy data. The mathematical mode and correlations have been applied to 10 tablets of pharmaceutical products ACP and Sedalgin.

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