

Determination of possible impurities in piracetam using FTIR spectroscopy

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Abstract

Piracetam (2-oxo-1-pyrrolidine acetamide (PAm)) may contain some structurally related impurities deriving from synthesis or degradation, mainly 2-oxo-1-pyrrolidine acetic acid (PAC). A rapid and sensitive Fourier transform infrared (FTIR) spectrophotometric method was developed for determination of possible impurities in piracetam. The results showed that the method was effective for the simultaneous determination of PAC in piracetam by FTIR spectrophotometry and may be a real alternative to HPLC. The apparent spectral resolution was first enhanced by using the Fourier self-deconvolution (FSD) method and the profiles were then fully deconvoluted by using a curve fitting procedure. FSD method followed by curve fitting allowed to evaluate quantitatively the areas under the pyrrolidine acetic acid peaks. Mixtures of known composition were used as standards to minimise errors due to the presence of both compounds in the same mixture. The detection limit of pyrrolidine acetic acid was estimated to be 0.1% with respect to piracetam. © 2000 Elsevier Science B.V. All rights reserved.

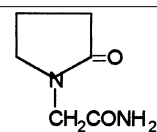
Keywords: Piracetam; FTIR spectroscopy; Deconvolution; Curve fitting

1. Introduction

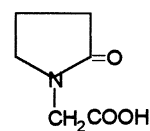
Piracetam (2-oxo-1-pyrrolidine acetamide (PAm)) is a drug clinically effective in the treatment of memory impairment, especially in elderly patients [1]. Pure PAm can be identified by using infrared spectroscopy [2]. The bulk drug may contain some structurally related impurities, deriving from the synthesis, such as 2-oxo-1-pyrrolidine acetic acid (PAC) and 2-oxo-1-pyrrolidine ethylacetic ester (PEs).

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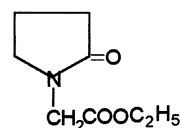
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P A m



P A c



P E s

These impurities are usually identified by the manufacturer by normal-phase TLC, using silica gel layers, developed with ethyl acetate–methanol (30:70), then exposed to iodine vapors [1]. The method lacks sensitivity and does not allow a quantitative analysis, furthermore the impurities can be identified only if they are present at a level greater than 0.5%.

Owing to the physicochemical properties of piracetam, lack of chromophores for UV-VIS determination and degradation under GC conditions, the analytical approach for its determination has been based on HPLC [1]. Recently Gagliardi et al. [3] have developed a method for the simultaneous analysis of piracetam and its impurities using HPLC. Although HPLC provides a sensitive and accurate procedure for determination of impurities, the method is time consuming and expensive.

In the last years Fourier transform infrared (FTIR) spectrophotometry combined with the appropriate software finds a very wide application in pharmacy due to its possibilities for solving many important problems. IR spectroscopy and IR techniques have well known advantages in comparison with other analytical methods, e.g. rapidity, cheapness and simplicity, non-destructive technique, quantitative analysis, in-process control during manufacturing of drugs, etc. [4]. IR spectroscopy secures simultaneously verification of drug identity and examination of drug test purity.

The aim of the present study was to investigate the possibilities of FTIR spectrophotometry for quantitative determination of impurities in piracetam.

2. Experimental

2.1. Instrumentation

FTIR spectra (KBr) were recorded, in the 3700–650 cm^{-1} range, on a Perkin–Elmer Spectrum 1000 FTIR spectrophotometer; resolution 2 cm^{-1} , sample thickness 1 mm. As a rule, from 128 to 256 spectra were collected and signal-aver-

aged for each sample. All the samples in this study were at appropriate concentration to absorb in a range where the Lambert–Beer law is obeyed. All spectra were normalized. Each spectrum was analyzed by second derivatization, Fourier self-deconvolution (FSD) and curve fitting. Before curve fitting spectra were subjected to FSD using a bandwidth of 6 cm^{-1} and a smooth factor of 5. Maxima found by second derivative spectroscopy and FSD were in agreement using these values. All spectra were transferred to a personal computer and subjected to a line shape analysis by the GRAMS-software [5]. This program allows appropriate base line corrections, deconvolution of highly overlapping bands and discrimination between Lorentzian, Gaussian, mixed Lorentzian–Gaussian and Voigtian band profiles. Curve fitting was performed using the original, non-resolution-enhanced spectra in the carbonyl region. The mixed Gaussian–Lorentzian curve fitting was then performed using the numbers and positions of the bands determined by the FSD method, derivative and difference spectra as starting parameters. Fitting procedure itself was carried out by iterative adjustment of the heights and half-width of the component bands with peak full width at half height (FWHH) of 6 cm^{-1} . The pyrrolidine acid content was calculated from the areas of the individual assigned bands and their fraction of the total area in the carbonyl region (1750–1600 cm^{-1}). The wavenumbers and areas of the fit bands were averaged and the standard deviation were calculated.

2.2. Reagents

All reagents were of analytical grade quality, unless otherwise specified.

Piracetam, pyrrolidine acetic acid and pyrrolidine acetic ester were produced by Pharmacia-AD, Bulgaria. The purity of the products was checked by HPLC according to the method proposed in the France Pharmacopeia [1].

2.3. Procedure for calibration curves

The test procedure uses a series of artificial binary mixtures containing increasing amounts of

PAC in the presence of PAm. Accurately weighed samples (± 0.00001 g) of PAm and PAC in the ratios from 99.5:0.5, 99:1, 95:5, 90:10, etc., were mixed and milled, and then 10 mg of each mixture made into a tablet with KBr (300 mg). The various measures are inspected for linearity, intercepts and detection limit. The mixed Gaussian–Lorentzian curve fitting gave the lowest errors and best sensitivity.

Laboratory batches of piracetam with known concentration of pyrrolidine acetic acid were produced in order to determine the accuracy, precision, linearity and range of test procedures.

3. Results and discussion

3.1. FTIR spectra

For a IR analysis, PAm can be assayed directly at 1696 cm^{-1} , but in combination with PAC, in which they usually are after incorrect synthesis, quantitative determination of PAC is not possible. This fact is due to the large overlap of the spectral bands of the two compounds. However, the FSD

method have spectral features that can be used for the simultaneous determination of these two components. In the present analysis we will be mainly concerned with the C=O stretching region, which is expected to be the most sensitive to the occurrence of structurally related impurities. The ester precursor could not be identified in the drug, because the reaction conditions by the synthesis yielded to the hydrolysis of PEs to PAC [6]. For this reason determination of PEs was not carried out.

Fig. 1 shows the FTIR spectra of pure PAm, 50% w/w mixture of PAm and PAC, and pure PAC. In the carbonyl stretching region of the FTIR spectra of PAm two peaks are usually observed: 1695 cm^{-1} for the amide carbonyl group and 1659 cm^{-1} for the cyclic ketone group [2]. The observed carbonyl band contours are complex composites: they consist of many overlapping component bands possible due to Fermi resonance, amide resonance, *cis-trans* isomerization or conformation [7,8]. Fortunately pyrrolidine acetic acid give rise to two carbonyl peaks centred at 1723 and 1631 cm^{-1} . As can be seen from Fig. 1b the presence of pyrrolidine acetic

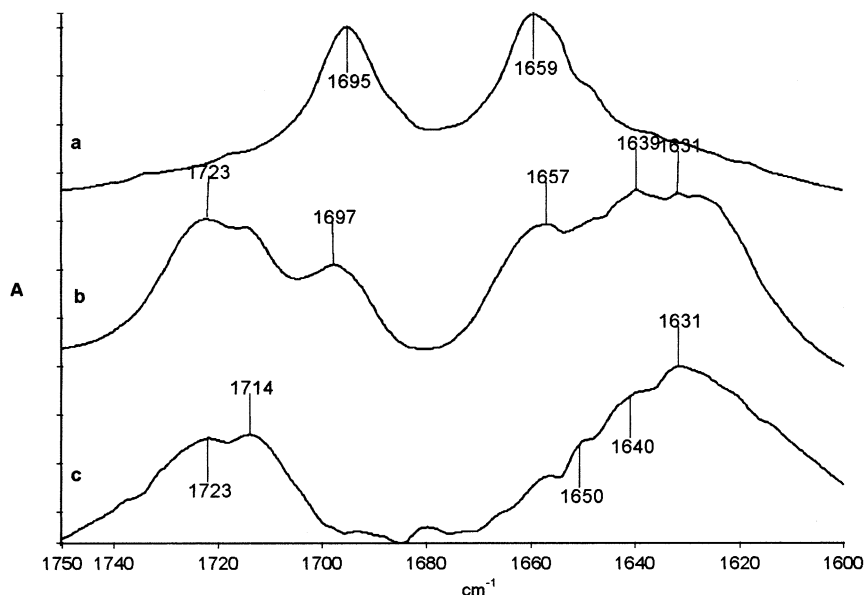


Fig. 1. FTIR spectra of (a) pyrrolidine acetamide, (b) 50% w/w mixture from pyrrolidine acetamide and pyrrolidine acetic acid, (c) pyrrolidine acetic acid.

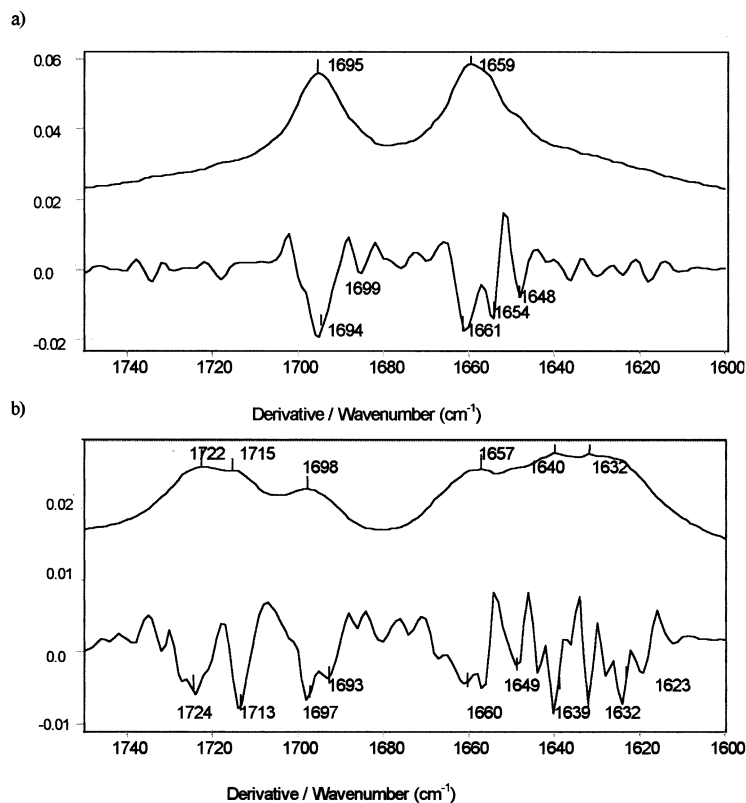


Fig. 2. Derivative spectra of (a) PAm, (b) 50% w/w mixture from PAm and PAc.

acid causes broadening not only at the amide peak, but also at the ketone peak. The effect was interpreted in terms of the occurrence of hydrogen bonding interactions involving the OH groups and the ketone carbonyl of PAc. For this reason we have chosen the whole carbonyl region for quantitative determination of the by-product pyrrolidine acetic acid.

It was informative to perform a second derivative analysis (GRAMS-software) in the carbonyl stretching region [9]. This method relies on the fact that the second derivative of the Gaussian or Lorentzian function, which can be used in most instances to represent the shape of infrared absorption curves, is itself a negative peak with two positive lobes; the sharpness of this peak, or its FWHH is approximately one third of the original function and this factor can be used to separate overlapping bands by double differentiation. The 'Savitsky–Golay' method was used for this pur-

pose. In Fig. 2 the second derivative spectra of PAm and a 50% w/w mixture of PAm and PAc in the carbonyl region are shown. Four well resolved negative peaks (Fig. 2a) are observed in the spectrum of pure PAm, 1694, 1661, 1654 and 1648 cm^{-1} . The derivative spectrum of the mixture (Fig. 2b) is complex and indicates the presence of another component. The exact location of their maxima is also obtained. Derivative analysis gave qualitative information only about possible impurities, but it is very useful to avoid over-deconvolution [9].

Another method which can yield useful information in this frequency range is spectral subtraction analysis. Further improvements in the detecting impurities are likely to occur as a result of a more general use of the strategy of difference infrared spectroscopy. In this approach, spectra of mixtures and pure compound are measured. These spectra are subsequently subtracted to yield

a difference spectrum that represents only the impurity, with the PAm absorption being removed. In fact, it was found that in the region of interest a remarkably ‘clean’ subtraction is obtained in the form of peaks at 1723 cm^{-1} and a complex peak centred at 1632 cm^{-1} (Fig. 3c). Difference spectrum shows that the presence of PAc can be monitored at the 1723 cm^{-1} band.

Both the spectral subtraction method and derivative analysis gave only qualitative information about possible impurities.

The FSD method has been developed for the determination of by-products of piracetam. The apparent spectral resolution was first enhanced by

using the FSD method and the profiles were then fully deconvoluted by using a curve fitting procedure. The FSD method consists of separating an assumed lineshape function from the experimental spectrum; the process reduces the bandwidth of the absorption, thereby improving the ‘apparent’ resolution [9]. One important feature which distinguishes FSD from derivative methods is that the integrated band areas are not altered by the process. Therefore, more information on the relative intensity of overlapping components is obtained at the expense of lineshape information [10,11].

FSD method followed by curve fitting allowed both qualitative evaluation of the presence of

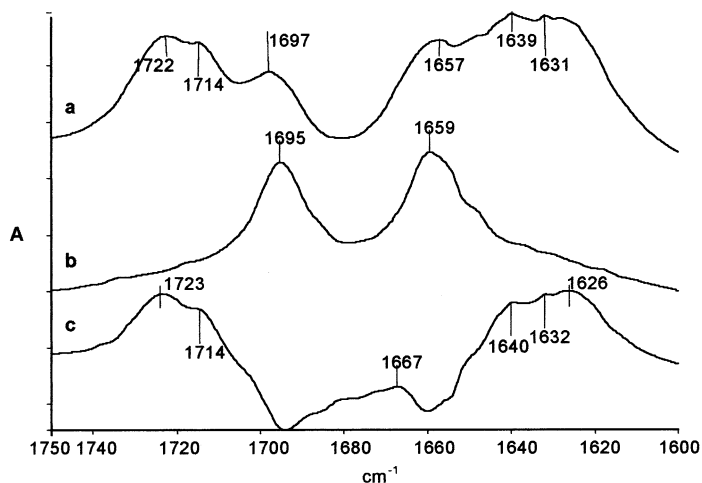


Fig. 3. (c) Difference spectrum from (a) 50% w/w mixture of PAm and PAc and (b) pure PAm.

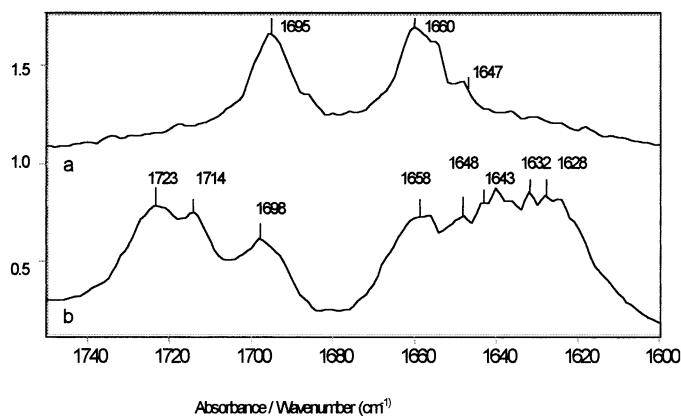


Fig. 4. Deconvolution spectra of (a) pure PAm and (b) 50% w/w mixture of PAm and PAc.

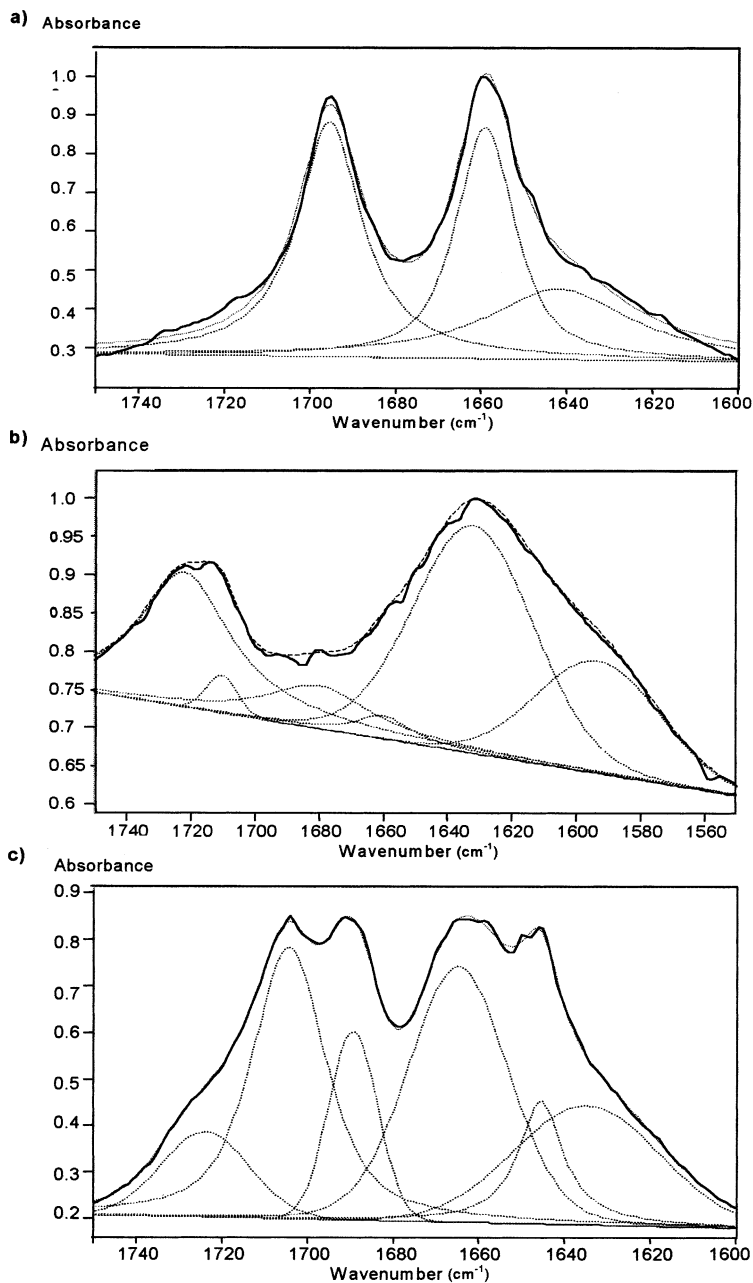


Fig. 5. Curve fitting of (a) PAm, (b) PAc (c) mixture PAm:PAc 99:1% w/w. —, Experimentally observed spectra; ---, curve fit; ····, calculated peaks.

pyrrolidine acetic acid and quantitative determination. The analysis of derivative (Fig. 2), difference (Fig. 3) and deconvolution (Fig. 4) spectra showed that the absorption at 1696 cm^{-1} was

specific for PAm and at 1723 cm^{-1} for PAc and gave the possibility for curve fitting procedure.

The areas under 1696 and 1723 cm^{-1} were selected to determine PAc (Fig. 5a, b and c). It is

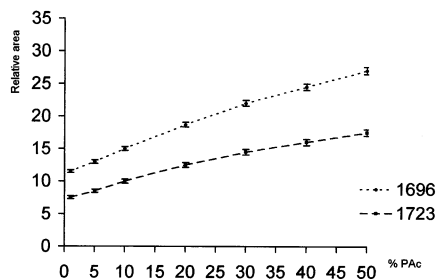


Fig. 6. Calibration curves.

assumed that Lambert–Beer's law is applicable to the absorption spectrum of the system investigated. The quality of the statistical significance of the fits were judged by their residuals and reduced χ^2 numbers [11]. This strategy enables us to obtain reliable spectral parameters of even strongly overlapping bands. We then determined the normalized areas of these bands for all mixtures investigated and found them to append linear on the mixing ratio (Fig. 6). This shows that the analyte's concentration ratios correspond to the mixing ratios of the artificial binary mixtures. In Fig. 6 the percentage of the total integrated absorbance in the carbonyl region due to the 1723 and 1696 cm^{-1} component is reported as a function of mixing ratios. The content of PAc can be monitored either by the increasing the area of the 1723 cm^{-1} peak or by the decreasing the area of the 1696 cm^{-1} peak.

Mathematically, the curve fitting analyses of overlapping band contours contains an element of subjectivity (initial choice of input parameters) and uncertainty. The result of automatically fitting with a large number of adjustable param-

eters is not necessary unique. For this reason the estimation of the input parameters by difference and derivative spectroscopy is very important. However, methods based on band narrowing, if used cautiously, provide a sensitive tool for monitoring possible impurities.

3.2. Statistical analysis of results [1,12,13]

Calibration graphs for PAc were made by calculating the relative areas of the 1723 and 1696 cm^{-1} peaks towards the total area of the carbonyl peaks in the region 1750–1600 cm^{-1} . The percentage of PAc is given according to the artificial binary mixtures described in the experimental part. Calibration curves at 1723 and 1696 cm^{-1} are given in Fig. 6. As could be seen in the plots of Fig. 6 in a wide range of concentrations (0.5 ÷ 50% PAc) the calibration curves were nearly linear. Nevertheless in the range of interest for pharmaceutical analysis the curves exhibited good linearity, which will be discussed later.

The statistical data obtained from the different calibration graphs in the concentration range 0.5 ÷ 50% PAc (0.05 ÷ 5 mg mg^{-1} mixture) are given in Table 1. The correlation coefficients of the regression equations are very close to unity. For routine analysis a graph constructed with five concentration levels within the range investigated was found to be sufficient as shown in the part linearity and range. Relative error does not exceed 2.6%.

It is true that the relative error of HPLS analysis is smaller $\pm 1.5\%$ [3]. But the average analysis time is about 20 min, without the preparation of the solutions and the apparatus. We will not

Table 1

Statistical data obtained from the calibration curves for equation $y = a + bx^a$

Data	1723 cm^{-1}		1696 cm^{-1}	
	Value	Confidence interval	Value	Confidence interval
Intercept (a)	7.6298	± 1.0412	11.4798	± 1.2049
Slope (b)	0.2099	± 0.0396	0.3263	± 0.0458
Correlation coefficient (r)	0.9923	–	0.9957	–
S.D. (σ)	0.281	–	0.3763	–

^a Significance level $\alpha = 0.01$.

Table 2
Precision of the analytical procedures — repeatability^a

No.	Amount found (mg mg ⁻¹)	Error (%)
1	0.2041	+2.05
2	0.2026	+1.30
3	0.2038	+1.90
4	0.2043	+2.15
5	0.2045	+2.25
Mean	0.2039	—
Interval ($\alpha =$ 0.01)	± 0.0017	—
$\sigma^{2*}10^7$	7.0375	—

^a Determined at 1723 cm⁻¹.

Table 3
Intermediate precision of the analytical procedures^a

No.	Amount found (mg mg ⁻¹)	Error (%)
1	0.2046	+2.3
2	0.2034	+1.7
3	0.2052	+2.6
4	0.2060	+3
5	0.2033	+1.65
Mean	0.2045	—
Interval ($\alpha =$ 0.01)	± 0.0027	—
$\sigma^{2*}10^6$	1.6875	—

^a Determined at 1723 cm⁻¹.

Table 4
Accuracy of the analytical procedure — linearity and range^a

Amount added (mg mg ⁻¹)	Amount found (mg mg ⁻¹)	Error (%)
0.102	0.104	+1.961
0.261	0.267	+2.298
0.525	0.533	+1.524
0.728	0.735	+0.962
1.342	1.358	+1.192
Correlation coeffi- cient (<i>r</i>)	0.9999	—
Intercept (<i>a</i>)	-0.0007	—
Confidence interval ($\pm \Delta a$)	± 0.0188	—
Slope (<i>b</i>)	1.006	—
Confidence interval ($\pm \Delta b$)	± 0.0256	—

^a Determined at 1723 cm⁻¹.

discuss here the cost of one HPLC analysis. The average analysis time for FTIR analysis is 5 min (one scan, 7 s) and does not need any expensive solvent, column, etc. It needs only an intelligent operator.

3.3. Precision — repeatability and intermediate precision

In order to test the precision of the proposed method, a determination of synthetic mixtures (98:2) according to the pharmacopoeia requirements was carried out. The amount of PAc added was 0.2 ± 0.003 mg mg⁻¹ and the content of PAm was 9.8 ± 0.005 mg mg⁻¹. Satisfactory results were obtained for the repeatability of the test procedures (Table 2).

Validation of the method [1,13] was accomplished by analysing mixtures of which were done in a blind fashion (the analyst did not know the concentration of the samples). The results are shown in Table 3. In the blind study (intermediate precision), accuracy and precision were also good as measured by the overall recovery and relative errors.

3.4. Linearity and range

For checking linearity and range of the method series samples of different batches were analyzed. The results are given in Table 4. The method gave concordant results with good reproducibility. Within the given range the results are proportional to the concentration (amount) of analyte in the sample. Statistical analysis confirms a probability of more than 0.99 in the range $0.1 \div 1.3$ mg mg⁻¹ for linearity, accuracy and precision of the analytical procedure. The detection limit of PAc was estimated to be 0.01 mg mg⁻¹ mixture.

4. Conclusions

The results demonstrated that the FSD method followed by curve fitting could be applied to the simultaneous determination of pyrrolidine acetamide and its by-product pyrrolidine acetic acid without significant mutual interference. This

method is sensitive, rapid and do not require any preliminary separation or treatment of the samples. The method could be used for monitoring of the synthesis too, if films from the reaction mixtures were prepared. This study shows that the FSD method followed by curve fitting give reliable results for analytes at low levels and may be an useful alternative to HPLC. Although HPLC is the official method of France Pharmacopoeia for the assay, the proposed FTIR FSD spectrophotometric method is fast, economical and suitable for the simultaneous determination of PAc and PAm and could be useful method for content uniformity tests. Reproducibility, accuracy and sensitivity of the method are satisfactory. The method may be considered for routine analysis of large number of samples of piracetam.

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