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Method Development for Quantification of Donor-Acceptor Complexes of Alverine Citrate and Tapentadol by Visible Spectrophotometry

Vijayalakshmi Rajendran*, Naga Sri Ramya Yerrapothini, Sasi Kiran Guntamukkala, Keziya Sugandha, Dhanaraju Magharla Dasaratha

GIET School of Pharmacy, Rajahmundry, Andhra Pradesh, India.

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ABSTRACT

In this present work two colorimetric methods were developed based on donor-acceptor complexes of alverine citrate (ALV) and tapentadol (TAP) with cobalt thiocyanate. These methods were developed on Perkin Elmer LAMBDA 25 UV–VIS spectrophotometer with 1cm quartz cells. The colored species formed are the coordination complexes of the drugs (electron donor) and the central metal atom of cobalt thiocyanate (electron acceptor) which is extractable into nitrobenzene from aqueous solution. The reaction conditions were optimized and validated to achieve maximum colour intensity. The colored complexes show maximum absorbance measured at 625 nm for both ALV and TAP. The absorbances were found to increase linearly with an increase in concentration which was corroborated by the calculated regression coefficients (0.9998-0.9999). Linearity was obeyed in the range of 100-600 µg/ml for both ALV and TAP, respectively. The molar absorptivity, sandell's sensitivity, LOD, LOQ and other validation parameters have been evaluated extensively as per ICH guidelines and all the parameters were found within the acceptance criteria for both methods. The proposed methods were proven to be more accurate, simple, precise and rapid by statistical validation of recovery studies and could be suitable for regular analysis.

INTRODUCTION

Alverine citrate (British Pharmacopoeia, 2008) chemically is N-Ethyl-N-(3-phenyl propyl)-benzene propanamine (Fig. 1) also called as spasmaverine used as an antispasmodic by directly acting on the muscle in the gut to relax. Tapentadol (Singh *et al*., 2013), chemically 3-[(1*R*, 2*R*)-3-(dimethylamino)-1-ethyl-2-methylpropyl]phenol hydrochloride (Fig. 2) is a centrally acting opioid analgesic. Literature is enriched with several techniques like HPLC and spectrophotometry for the determination of TAP (Asha *et al*., 2012; Babu and Madhu, 2012; Deepti and Pawan, 2013; Indira *et al*., 2013; Krishanmurthy *et al*., 2014; Neol *et al*., 2009; Omkar and Priti, 2012; Rizwana *et al*., 2014; Suresh et at., 2013) and ALV(Vijayalakshmi *et al*., 2014; Ghosh *et al*., 2010; Rahul *et al*., 2011; Kumar *et al*., 2013). The reported methods suffer from one or more disadvantages such as narrow linear response, lack of sensitivity and selectivity and usage of expensive reagents. The need for sensitive, cost effective and reliable spectrophotometric methods for the selected drugs is thus obviously recognized. Spectrophotometry is by far the instrumental technique of choice in the laboratories of under developed and developing nations for the quantification of drugs owing mainly to its simplicity, high sensitivity and selectivity and often demanding low cost equipment.

The tertiary amino group present in both drugs has been exploited for quantification of the drugs by the chemical reaction with cobalt thiocyanate to give the coordination complex. The coordination complexes were soluble in organic solvent and could be separated and quantified. The present work was aimed to explore the significance of donor-acceptor complexes with cobalt thiocyanate which was not reported earlier for the quantitative analysis of ALV/TAP and to validate the methods accordingly with the ICH guidelines.

* *Corresponding Author*
Email: vijayalakshmigsp@gmail.com

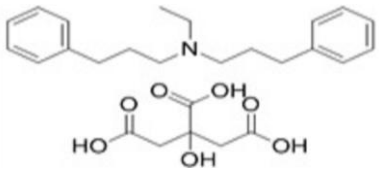

Fig. 1: Chemical Structure of ALV.

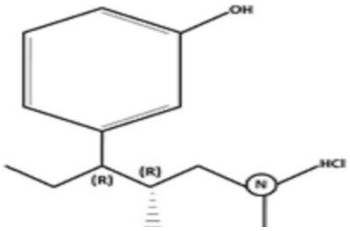

Fig. 2: Chemical Structure of TAP.

EXPERIMENTAL

Instruments

Double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer with 1 cm matched quartz cells was used for spectral measurements. Samples were weighed using Sartorius electronic balance.

Chemicals

Pharmaceutical grade ALV and TAP was graciously donated by Aurobindo Pharma Ltd, Hyderabad. Cobalt thiocyanate, nitrobenzene and hydrochloric acid of AR grade were used for the experimental work. Double distilled water was used in the preparation of solutions. All the preparations were prepared afresh daily.

Preparation of cobalt thiocyanate (0.25M)

Accurately 7.25 g of cobalt nitrate and 3.8 g of ammonium thiocyanate was weighed and taken together in 100 ml volumetric flask, dissolved and diluted to the final volume with distilled water.

Preparation of 0.2M hydrochloric acid

Accurately 1.7 ml of concentrated hydrochloric acid was diluted to 100 ml with distilled water to get 0.2 M HCl solution.

Preparation of standard stock solution for estimation of ALV

Accurately 25 mg of ALV was weighed and transferred to a 25 ml volumetric flask, dissolved and diluted to final volume with water. The resulting solution has a concentration of 1mg/ml.

Preparation of standard stock solution for estimation of TAP

Accurately 25 mg of TAP was weighed and transferred to a 25 ml volumetric flask, dissolved and diluted to final volume with water. The resulting solution has a concentration of 1mg/ml.

Procedure for calibration plot of ALV

Into a series of 50 ml separating funnels appropriate volume of 0.5-3.0 ml (1ml=1mg/ml) of ALV was pipetted out and 0.5 ml of HCl (0.2M), 0.3 ml of (0.25M) cobalt thiocyanate reagent was added, mixed well followed with 5 ml of nitrobenzene and mixed well, allow to separate the two layers. The absorbance of the greenish blue coloured organic layer was measured at 625 nm against reagent the blank. The amount of ALV present in the sample solution was computed from its calibration curve.

Procedure for calibration plot of TAP

Into a series of 50 ml separating funnels appropriate volume of 0.5-3.0 ml (1ml=1mg/ml) of TAP was pipetted out and 1 ml of HCl (0.2M), 1 ml of (0.25M) cobalt thiocyanate reagent was added and mixed; followed by 5 ml nitrobenzene and shaken thoroughly, allowed to separate the two layers.

The absorbance of the greenish blue coloured organic layer was measured at 625 nm against reagent blank prepared similarly in each case. The amount of TAP present in the sample solution was computed from its calibration curve.

Assay procedure for ALV

Twenty tablets of commercial samples (Spasverin 60 mg) of ALV were accurately weighed and powdered. Tablet powder equivalent to 25 mg of ALV was weighed and dissolved in 25 ml water, filtered and the procedure was carried out as mentioned above in section 2.3.1.

Assay procedure for TAP

Twenty tablets of commercial samples of TAP (Tapenta 100 mg) were accurately weighed and powdered. Tablet powder equivalent to 25 mg was weighed and dissolved in 25ml water, filtered and the procedure was carried out as mentioned above in section 2.3.2.

RESULTS AND DISCUSSION

Optimization of the Method

The method was optimized by selecting the proper chromogen, concentration of the reagent, order of addition, selection of the wavelength, linearity and stability of the coloured product.

Selection of the chromogenic reagent

Several chromogenic reagents like cobalt thiocyanate, citric acid/acetic anhydride, N-bromosuccinimide has been studied and cobalt thiocyanate has been proved to be a valuable chromogenic reagent for the detection and determination of ALV/TAP based on the sensitivity of the method.

The colored product was formed by the coordination complex of the drug (electron donor) and the central metal atom of cobalt thiocyanate (electron acceptor) which was extractable into nitrobenzene from aqueous solution, hence could be separated and quantified.

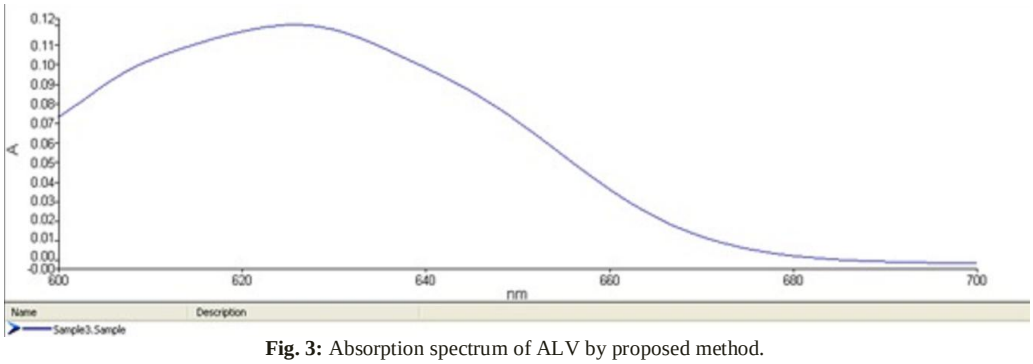

Fig. 3: Absorption spectrum of ALV by proposed method.

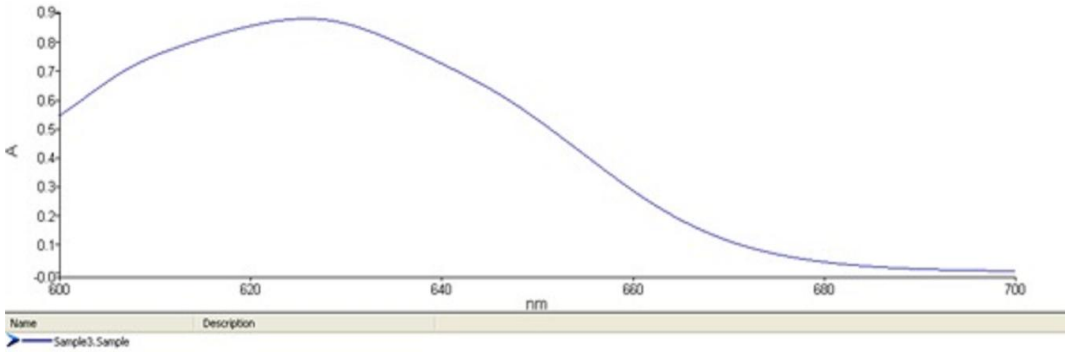

Fig. 4: Absorption spectrum of TAP by proposed method.

Effect of cobalt thiocyanate concentration

It was studied by treating the fixed volume of ALV/TAP and HCl concentration and in-turn varying the volume of cobalt thiocyanate from 0.1-2.0 ml. The results for both methods were depicted in **Table 1**.

Table 1: Order of addition/concentration of reagents for the proposed methods.

0.5 ml (0.2M)HCl+ 0.3 ml (0.25M) Cobalt thiocyanate + ALV + nitrobenzene
1 ml (0.2M) HCl +TAP + 1 ml (0.25M) Cobalt thiocyanate + nitrobenzene

Effect of time/temperature on reaction

The effect of time and temperature on the formation of the coloured complex was studied for the both methods. The complex formation was complete in 5-7 min time interval at room temperature for both the drugs. Fig. 3 and 4 represents the absorption spectrum of ALV and TAP, respectively.

Method validation

All the methods were validated for accuracy, precision, linearity, LOD, LOQ, ruggedness and robustness and the results were found to be satisfactory.

Linearity and range

At the described experimental conditions for ALV/TAP standard calibration curves were constructed by plotting an increase in absorbance with concentration (Fig 6 and 7). A linear correlation was found between absorbance and concentration of ALV/TAP as shown in **Table 2** and all the parameters regarding linearity were given in **Table 3.**

Table 2: Linearity data of absorbance against concentration.

ALV		TAP	
Conc (µg/ml)	**Absorbance**	**Conc (µg/ml)**	**Absorbance**
100	0.18	100	0.30
200	0.35	200	0.58
300	0.52	300	0.85
400	0.68	400	1.13
500	0.85	500	1.42
600	1.02	600	1.71

Table 3: Optical and regression parameters.

Parameters	**ALV**	**TAP**
λ max, nm	625	
Beer's law range (µg/ml)	100-600	
Molar absorptivity (L.mole^{-1} .cm^{-1})	1.7×10^5	2.8×10^5
Sandell's sensitivity (µg/cm^2)/0.001 absorbance unit)	4×10^5	4×10^5
LOD, µg/ml	10.31	9.65
LOQ, µg/ml	31.24	29.27
Slope(m)	0.001689	0.002829
Intercept (b)	0.0075	0.007143
Correlation coefficient (r)	0.9998	0.9999

The statistical parameters given in the regression equation were calculated from the calibration graphs. The high values of the regression coefficients and low values of y-intercepts of the regression equations, proved the linearity of the calibration curves.

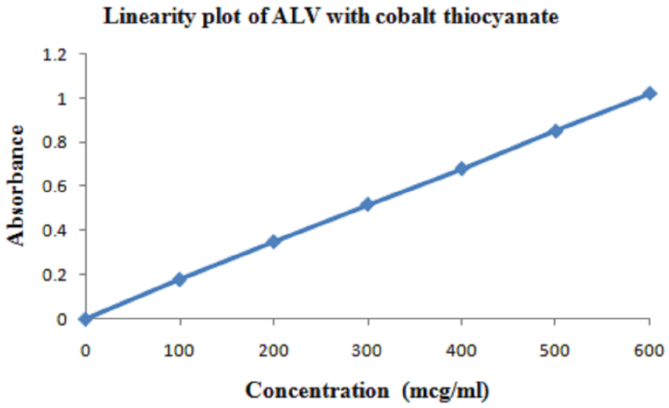

Fig. 5: Linearity plot of ALV.

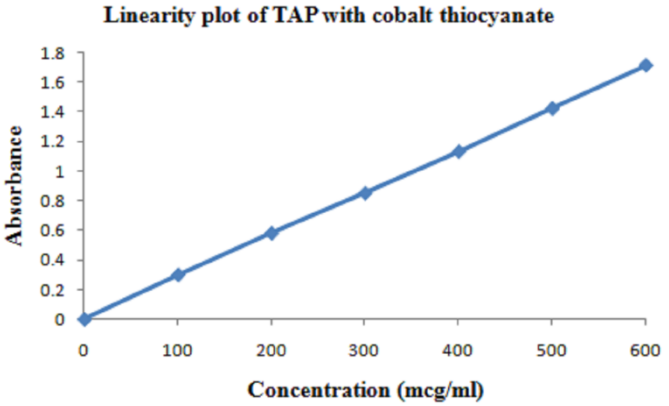

Fig. 6: Linearity plot of TAP.

Precision

The precision of the proposed methods was assessed by determining the relative standard deviation (RSD) of six replicate analyses at 80% level of ALV/TAP in linearity range. The low % RSD of the intraday and interday repeatability studies corroborates precision of the method. **Table 4** represents the results of precision studies.

Table 4: Results of precision studies.

Parameter	ALV		TAP	
	Intra day n=6	Inter day n=6	Intra day n=6	Inter day n=6
Conc	400 µg/ml		400 µg/ml	
Mean abs	0.68	0.675	1.13	1.131
SD	0.006325	0.010488	0.006325	0.007528
% RSD	0.93	1.55	0.55	0.66

Robustness

Robustness was checked by narrow alteration of the optimized parameters and the % RSD was 0.4 ± 0.12 and 0.6 ± 0.04 for ALV and TAP, respectively which was satisfactory.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined by analysing progressively lower concentrations of standard solution using optimized conditions and the results were presented in Table 3.

Accuracy

The validity and accuracy of the proposed methods were further assessed by recovery studies using the standard addition technique. For this purpose, a known amount of pure drug at three different levels was spiked to the fixed and known quantity of pre analysed formulation samples and the nominal value of the drug was estimated by the proposed methods. The results given in **Table 5** establish that the methods were reproducible by low SD and %RSD. No interference was evidenced from the commonly encountered formulation excipients.

Table 5: Results of accuracy studies of ALV and TAP by proposed methods

Drug	Drug in formulation (µg)	Std added (µg)	Amt Found (µg)	%Recovered	%RSD N=3
	400	200	598.42	99.73	0.135
AL	400	400	797.80	99.72	0.066
V	400	600	997.38	99.73	0.093
	400	200	597.52	99.58	0.112
TAP	400	400	797.03	99.62	0.145
	400	600	997.28	99.72	0.137

Application of the proposed methods to formulations

To evaluate the proposed methods, they were applied to the determination of ALV/TAP in commercial formulations. The recoveries are close to 100%, indicating that there is no serious interference in samples. The good agreement between these results and known values indicate the successful applicability of the proposed methods for the determination of ALV/TAP in formulations. The results are given in **Table 6**.

Table 6: Assay results of ALV/TAP.

Formulations	Label claim(mg)	Amount found(mg)	% Recovery N=3
Spasverin (tablet)	60	59.83	99.71
Tapenta (tablet)	100	99.93	99.93

CONCLUSION

Two new, cost effective, simple and sensitive visible spectrophotometric methods, using cobalt thiocyanate were developed for the quantitation of ALV and TAP in bulk and in pharmaceutical formulations. The developed methods were also validated. From the statistical data, it was found that the proposed methods were accurate, precise and reproducible and can be successfully applied to the analysis of the same and could make a better alternative to the existing methods.

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