Separation of Tramadol and Paracetamol in Pharmaceutical Formulations Using Thin Layer Chromatography with Sustainable Solvents Aligned to Pfizer's Green Chemistry Guidelines"

Rekha Kumari Jain[1], **Chandni Kirpalani** [2], **Rekha Nair** [3]
[1] *Poornima University, Jaipur, India,Email: Rekha.jain1304@gmail.com*
[2] *Poornima University, Jaipur, India,Email: registrar@poornima.edu.in*
[3]*Poornima College of Engineering, Jaipur, India, rekhanair@poornima.org*

Abstract- *A new, economic, eco-friendly and rapid thin layer chromatographic method was developed and validated for simultaneous detection and separation of Tramadol HCl and Paracetamol in pharmaceutical dosage forms. Forty - five sustainable solvent systems were examined from which only ten solvent systems which were the various combinations of Ethyl Acetate & Methanol, Methanol & Toluene and Ethyl Acetate, Methanol & Acetic acid, were found to be most suitable as in these systems most compact spots of Paracetamol and tramadol were obtained. The method employed TLC aluminium plates precoated with silica gel 60F254 as the stationary phase. UV detection was performed at a wavelength of 254 nm. It was also found that iodine fuming technique is the best visualising method for examining the TLC chromatograms of both drugs prior to subsequent instrumental analysis. Here in used solvents, Ethyl Acetate & Methanol are of preferred category and Toluene & Acetic acid are of usable category as per Pfizer's solvent selection guide. So here we are trying to move towards the world of green chemistry.*

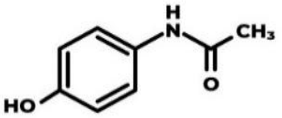
Keywords – *Paracetamol, Tramadol, pharmaceutical formulations, retardation factor, mobile phase, Iodine fuming, visualisation method*

INTRODUCTION

Paracetamol, N-(4-hydroxyphenyl) ethanamide (Figure. 1) is a widely used analgesic and antipyretic for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of severe pain (such as postoperative pain). [1]

Tramadol HCl is a synthetic codeine analogue that is a weak μ-opioid receptor agonist. It is used as an oral non-steroidal anti-inflammatory drug with good analgesic and tolerability profile in various painful conditions.[2]

Structure of Paracetamol



(Figure. 1)

Tramadol [2-(dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol] (Figure.2) has two stereo--genic centres at the Cyclohexane ring.[2] Literature survey revealed that various analytical methods like spectrophotometric [3–6], HPLC [6–14], GC [15] and HPTLC [16–21] have been reported for the determination of Tramadol HCl and paracetamol either individually or combination with some other drugs. Tramadol HCl and paracetamol are available in combined dosage forms. Most methods reported in the literature for the simultaneous determination of TMD and PAR in formulations by using HPLC. However, there is lack Structure of Tramadol (Figure. 2)

of such equipment in many resources limited countries. In poor countries, where such equipment is not available, the high costs of HPLC grade solvents and columns and the lack of the possibility to analyse many samples simultaneously, significantly affect timely release of laboratory results for action. Therefore, alternative methods are needed to facilitate and increase the speed of analysis, with relatively few costs.

In the present study an attempt has been made to analyse paracetamol and Tramadol pharmaceutical preparations(tablets) by thin layer chromatography using sustainable solvents as per Pfizer's Green

Chemistry Guidelines. In 2008, The scientists of Pfizer (Pfizer is pharmaceutical company of US) refined a list of solvents in which solvents are grouped as their acceptance to sustainability concepts. It is very helpful in solvent selection for any application.[22].

Preferred Solvents	Usable Solvents	Undesirable Solvents
Methanol	Cyclohexane	Pentane
Ethanol	Methyl cyclohexane	Hexane
1-Propanol	Heptane	Di isopropyl ether
2- Propanol	Isooctane	Diethyl ether
Butanol	t - butyl methyl ether	Dichloromethane
t - Butanol	Acetonitrile	chloroform
Ethyl acetate	methyl tetrahydrofuran	Di methyl formamide
Isopropyl acetate	Tetrahydrofuran	N-methyl Pyrrolidinone
Acetone	Dimethyl sulfoxide	Pyridine
Ethyl Methyl Ketone	Acetic acid	Dioxane
	Dimethyl acetate	Benzene
	Ethylene glycol	Carbon tetra chloride
	Xylene	
	Toluene	

Table – 1 Pfizer's solvent selection guide [22]

Method Development

Isolation and purification of the drugs from commercial formulations - The paracetamol tablet (1 tablet containing 500 mg of paracetamol) was powdered. The powder was shaken with HPLC methanol(30ml) in a 100ml beaker. The solution was filtered. The residue left in the beaker, was again extracted with HPLC methanol and filtered. The combined filtrate was left at room temperature until crystals appeared. The crystals were dried. The IR and mass spectra were recorded.

The same procedure was repeated for extraction of Tramadol from commercial formulations.

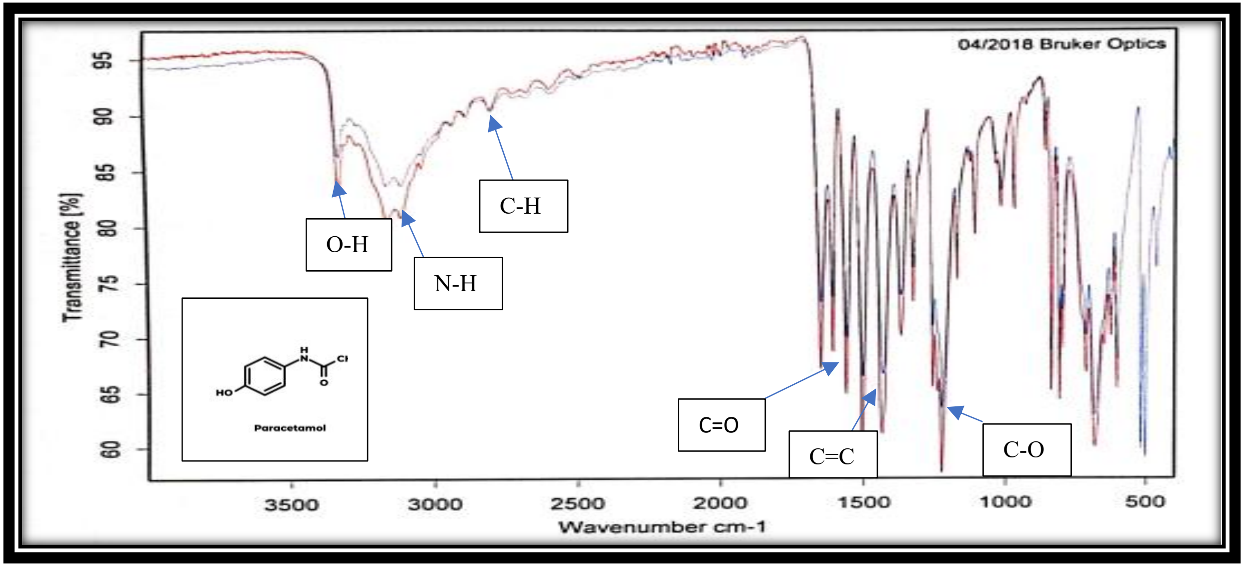
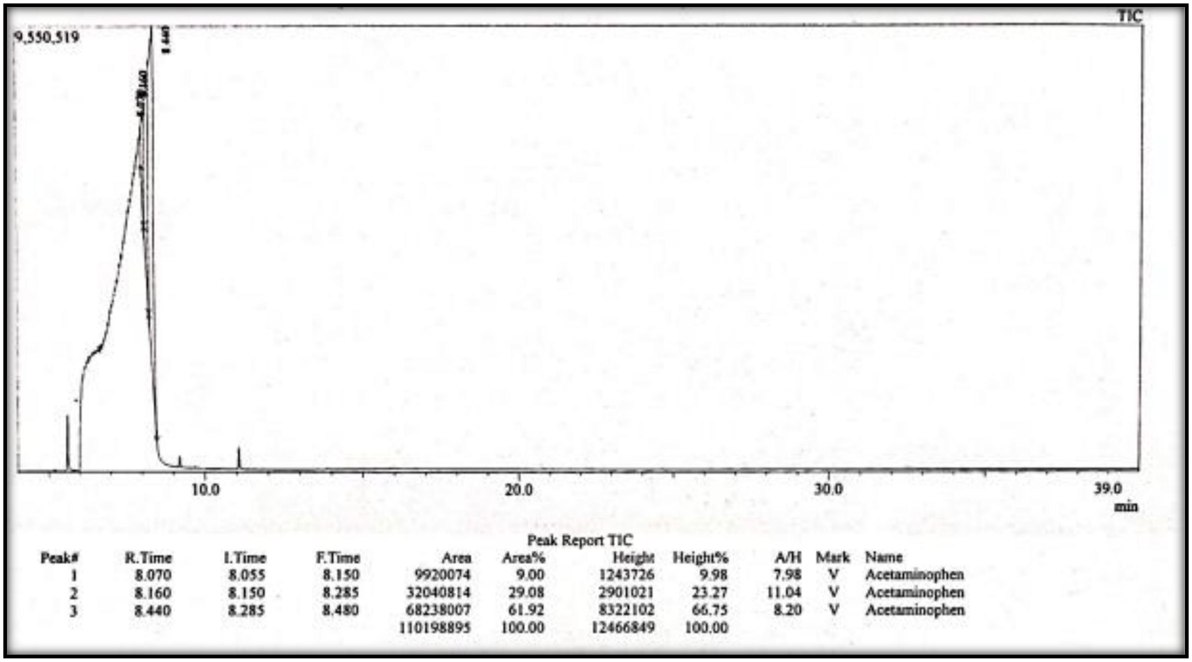


Figure – 3 Infrared spectrum of control Paracetamol

s.no.	Wave number (cm^{-1})	Functional group	Vibration Type
1	– 3500	-OH (Phenol)	O-H Stretch (broad)
2	3100 -3300	-NH (Amide)	N-H Stretch
3	1650	C=O (Amide)	C=O Stretch (Strong)
4	1500 - 1600	Benzene ring	C=C Aromatic Stretch
5	1300 -1350	C - N (Amide)	C-N Stretch
6	1200 -1250	C – O (Phenol)	C-O Stretch
7	750 - 850	C-H Aromatic	Out of plane bending

Table – 2 - Showing IR absorptions of Paracetamol



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    3    8.440    8.285    8.480    68238007   61.92     8322102    66.75    8.20    V   Acetaminophen
                                   110198895  100.00    12466849   100.00
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Figure – 4 Gas Chromatogram of control Paracetamol

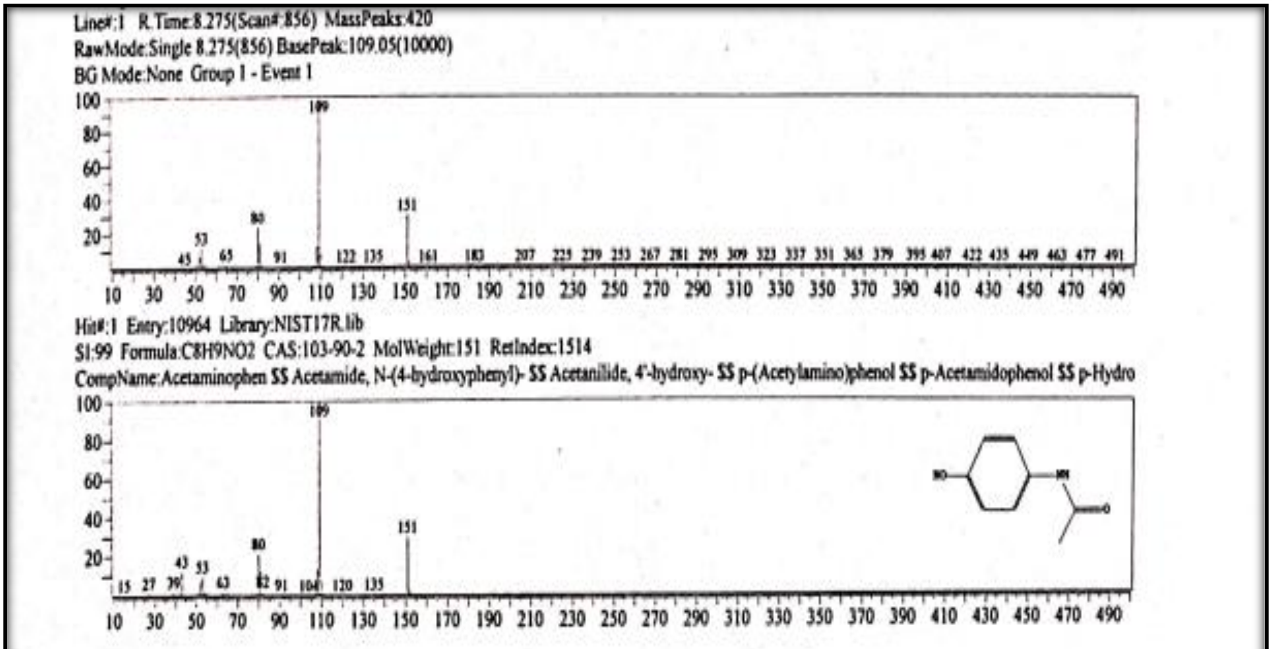

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Figure – 5 Electron Ionization (EI) mass spectrum of control Paracetamol

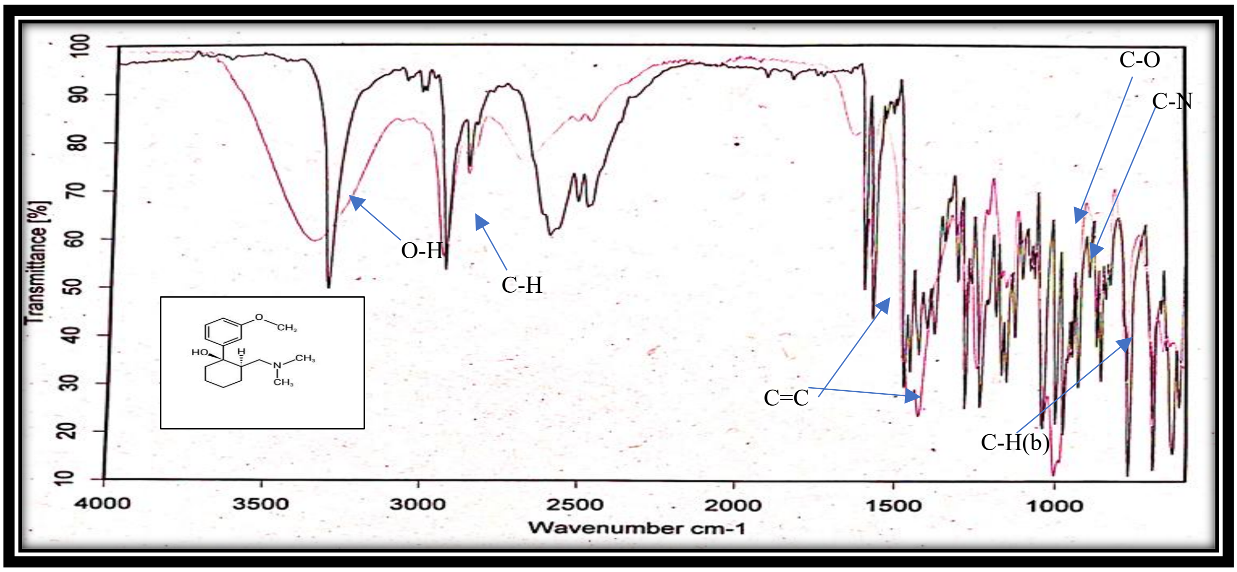
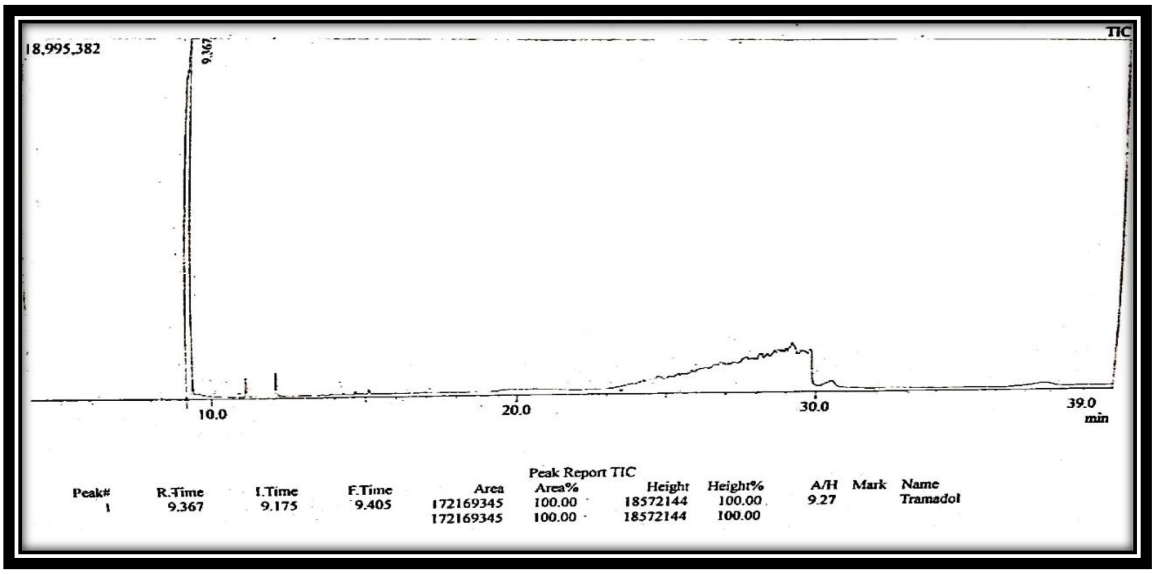


Figure – 6 Infrared spectrum of control Tramadol

s.no.	Wave number (cm^{-1})	Functional group	Vibration Type
1	3350– 3300	-OH	O-H Stretch (broad)
2	2950 -2850	-CH (Aliphatic) alkyl	C-H Stretch
3	1610 -1600	C=C (Aromatic)	C=C Stretch (Aromatic ring vibrations)
4	1500 - 1450	Aromatic skeletal vibrations	C=C Aromatic Stretch
5	1250 -1000	C - O (Ether or Alcohol)	C-O Stretch
6	1100 - 1020	C-N (Tertiary amine)	C-N Stretching
7	750 - 700	C-H Aromatic	Out of plane bending

Table – 3 - Showing IR absorptions of Tramadol



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				172169345	100.00	18572144	100.00			

Figure – 7 Gas Chromatogram of control Tramadol

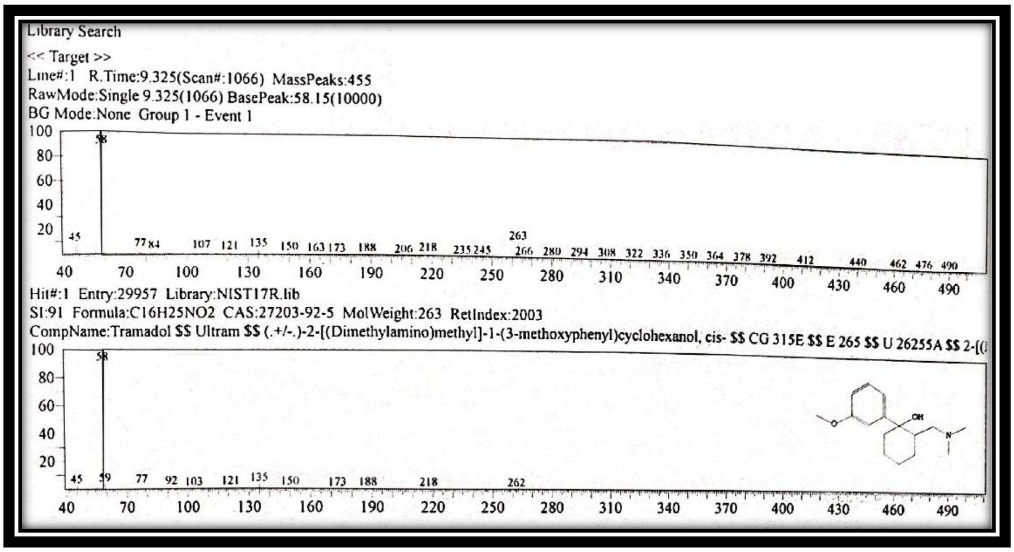

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Figure – 8 Electron Ionization (EI) mass spectrum of control Tramadol

Preparation of solutions – The purified compounds i.e. Paracetamol and Tramadol were used as standards and stock solutions in HPLC methanol were prepared. At the same time sample solutions from tablet available in combined dosage form (Paracetamol and Tramadol) was also prepared by dissolving tablets in methanol(30ml) and filtering it. The filtrate so obtained had two components.

Application of Thin Layer Chromatography for the separation of P and T – Forty-five solvent systems were tried on the drug samples [standard samples of Paracetamol (P)& Tramadol (T) as well as pharmaceutical formulation(P+T)] to find out the most suitable solvent system that could be applied universally for the thin layer chromatographic separation of all P+T preparations. The forty -five solvent systems (mobile phases) used in present study have been given in Table -1. Thin layer chromatographic plates (20 cm × 20 cm) were used in present study and these plates were cut down in 10 cm × 10 cm plates with the help of scale and Alpin. An aliquot amount of samples were then applied manually on the TLC plates using a fine capillary tube. The samples (P, T and P+T) were applied 1.5 cm above the base of plate. The spots were allowed to dry at room temperature. A solvent chamber was saturated using various solvent systems. Generally, 20 ml mixture of solvents was used as mobile phase. The spotted TLC plates were placed in saturated solvent chamber and the chamber was covered with lid. The solvent front was allowed to migrate to a distance of 7cm above the origin. Developed plates were taken out and dried at room temperature. Samples were visualised in under 254 nm, 366 nm and with Iodine fuming method, and their respective Rf (Retardation factor) were calculated using the following equation:

Rf = Distance travelled by solute from origin / Distance travelled by solvent from origin

TABLE-4 (LIST OF FORTY- FIVE SOLVENT SYSTEMS AND RF OF P AND T IN THESE SOLVENT SYSTEMS)

S.No.	Solvent System codes	Solvent systems	Mobile phase	Rf of Paracetamol	Rf of Tramadol	In combination tablet	
						Rf of Paracetamol	Rf of Tramadol
1	I	Toluene	100%	0.0	0.0	0.0	0.0
2	II	Cyclohexane	100%	0.0	0.0	0.0	0.0

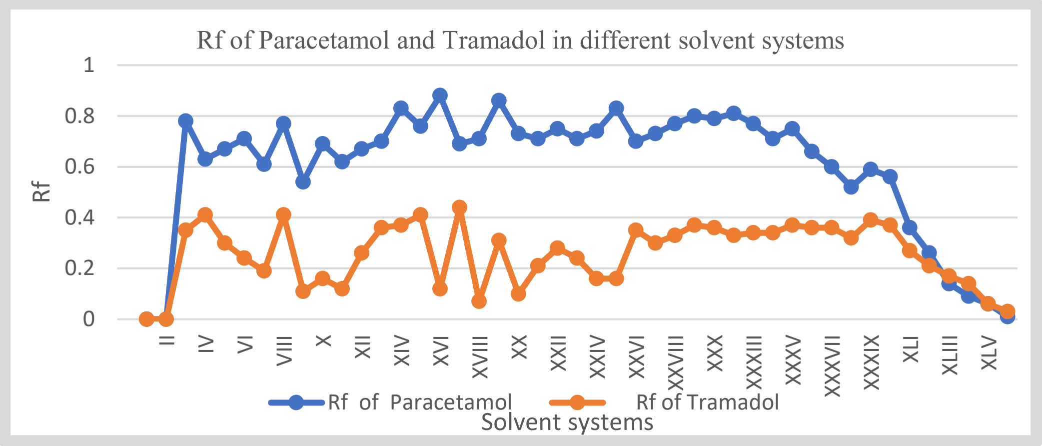
3	III	Acetone	100%	0.78	0.35	0.72	0.22
4	IV	Ethanol	100%	0.63	0.41	0.59	0.24
5	V	2-Propanol	100%	0.67	0.3	0.63	0.24
6	VI	1-Propanol	100%	0.71	0.24	0.64	0.2
7	VII	1-Butanol	100%	0.61	0.19	0.61	0.1
8	VIII	Methanol	100%	0.77	0.41	0.63	0.33
9	IX	Ethyl Acetate	100%	0.54	0.11	0.56	0.09
10	X	Ethyl Acetate: Methanol	18:2	0.69	0.16	0.67	0.14
11	XI	Ethyl Acetate: Methanol: toluene	18:1:1	0.62	0.12	0.62	0.12
12	XII	Ethyl Acetate: Methanol	16:4	0.67	0.26	0.64	0.21
13	XIII	Ethyl Acetate: Methanol	14:6	0.7	0.36	0.66	0.27
14	XIV	Ethyl Acetate: Methanol	12:8	0.83	0.37	0.8	0.33
15	XV	Ethyl Acetate: Methanol: toluene	12:8.5:2.5	0.76	0.41	0.76	0.27
16	XVI	water	100%	0.88	0.12	0.88	0.05
17	XVII	Acetic acid	100%	0.69	0.44	0.69	0.44
18	XVIII	Ethyl Acetate: Methanol: Acetic acid	18:3:1	0.71	0.07	0.71	0.07
19	XIX	Ethyl Acetate: Methanol: Acetic acid: water	18:3:1:2	0.86	0.31	0.86	0.31

20	XX	Ethyl Acetate: Methanol: Acetic acid	16:4:1	0.73	0.1	0.76	0.1
21	XX1	Ethyl Acetate: Methanol: Acetic acid	16:8:1	0.71	0.21	0.73	0.17
22	XXII	Ethyl Acetate: Methanol: Acetic acid	12:8:1	0.75	0.28	0.75	0.28
23	XXIII	Ethyl Acetate: Methanol: Acetic acid: Toluene	16:4:1:1	0.71	0.24	0.68	0.2
24	XXIV	Ethyl Acetate: Methanol: Acetic acid	20:4:1	0.74	0.16	0.74	0.09
25	XXV	Ethyl Acetate: Methanol: Acetic acid	20:5:1	0.83	0.16	0.83	0.14
26	XXVI	Methanol: Toluene	19:1	0.7	0.35	0.66	0.34
27	XXVII	Methanol: Toluene	18:2	0.73	0.3	0.65	0.27
28	XXVIII	Methanol: Toluene	17:3	0.77	0.33	0.67	0.31
29	XXIX	Methanol: Toluene	16:4	0.8	0.37	0.74	0.36
30	XXX	Methanol: Toluene	15:5	0.79	0.36	0.79	0.34
31	XXX1	Methanol: Toluene	14:4	0.81	0.33	0.84	0.34
32	XXXII	Methanol: Toluene	14:6	0.77	0.34	0.8	0.31

33	XXXIII	Methanol: Toluene	13:7	0.71	0.34	0.71	0.3
34	XXXIV	Methanol: Toluene	12:8	0.75	0.37	0.77	0.36
35	XXXV	Methanol: Toluene	11:9	0.66	0.36	0.69	0.36
36	XXXVI	Methanol: Toluene	10:10	0.6	0.36	0.63	0.33
37	XXXVII	Methanol: Toluene	9:11	0.52	0.32	0.60	0.30
38	XXXVIII	Methanol: Toluene	8:12	0.59	0.39	0.61	0.34
39	XXXIX	Methanol: Toluene	7:13	0.56	0.37	0.57	0.34
40	XL	Methanol: Toluene	6:14	0.36	0.27	0.41	0.24
41	XLI	Methanol: Toluene	5:15	0.26	0.21	No separation	
42	XLII	Methanol: Toluene	4:16	0.14	0.17	No separation	
43	XLIII	Methanol: Toluene	3:17	0.09	0.14	No separation	
44	XLIV	Methanol: Toluene	2:18	0.06	0.06	No separation	
45	XLV	Methanol: Toluene	1:19	0.01	0.03	No separation	

RESULTS AND DISCUSSION

In the present study, standard sample of Paracetamol, standard sample of Tramadol and pharmaceutical combination of Paracetamol and Tramadol were analysed using thin layer chromatography. Forty-five solvent systems (Mobile phases) were tried for the separation of Paracetamol and Tramadol in pharmaceutical formulation. To identify the optimal solvent system, initial TLC plate development was performed using individual solvents selected from the preferred and desirable categories of Pfizer's solvent table. By comparing the Rf of P and T in different single solvents, the different combinations of solvents were tried to find the best mobile phases for the analysis of P and T. And by this, ten mobile phases were found in which compact spots of P and T were obtaining. Developed thin layer chromatographic plates were examined under 366nm, 254 nm and with Iodine fuming method. Separated spots of Paracetamol and Tramadol were visible under 254nm UV light and with iodine fumes but not visible under 366nm UV light. The separated spots produced brown colour after reacting with Iodine fumes. In mobile phases I & II, the spots of studied drugs did not move and remain on base line. The mobile Phases XLI to XLV were failed to separate Paracetamol and Tramadol in pharmaceutical formulation because of less polarity of these Mobile phases so very less movement was taking place of studied drugs.

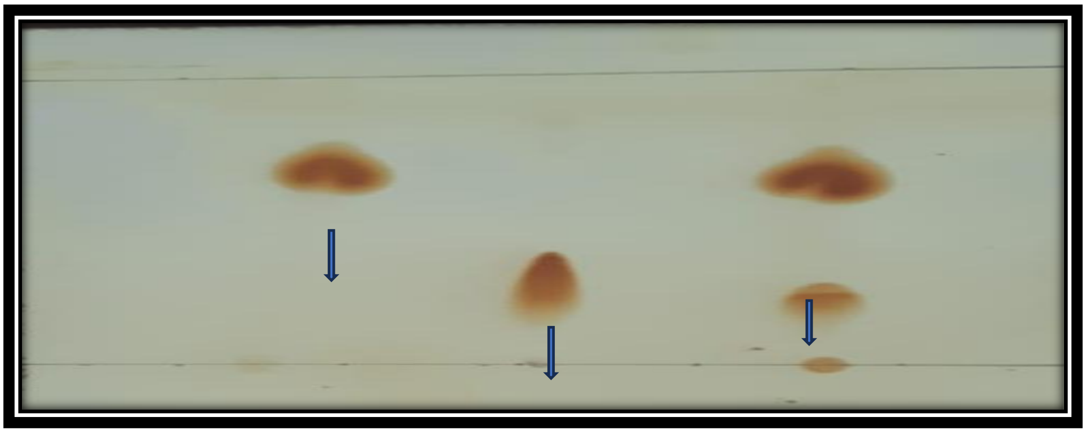


Graph -1 (showing Rf values of P and T in different solvent systems)

Standard P Standard T (P+T)

Figure 9 - photograph of the actual chromatogram demonstrates the lack of separation between paracetamol and tramadol when using mobile phase XLII.

In other studied mobile phases separation of paracetamol and Tramadol was taking place (graph -1) but compact spots of Paracetamol and Tramadol were not obtaining in all. In the solvent systems X, XII, XX, XXVI, XXVII, XXVIII, XXIX, XXXI, XXXIX, and XL compact spots of both P and T were obtaining. In solvent system XII more compact spots were obtaining compare to solvent system XIII. It could be seen in photographs 4 and 5



Standard P Standard T (P+T)

Figure 10 - Photograph of actual chromatogram showing separation of Paracetamol and Tramadol using mobile phase XII

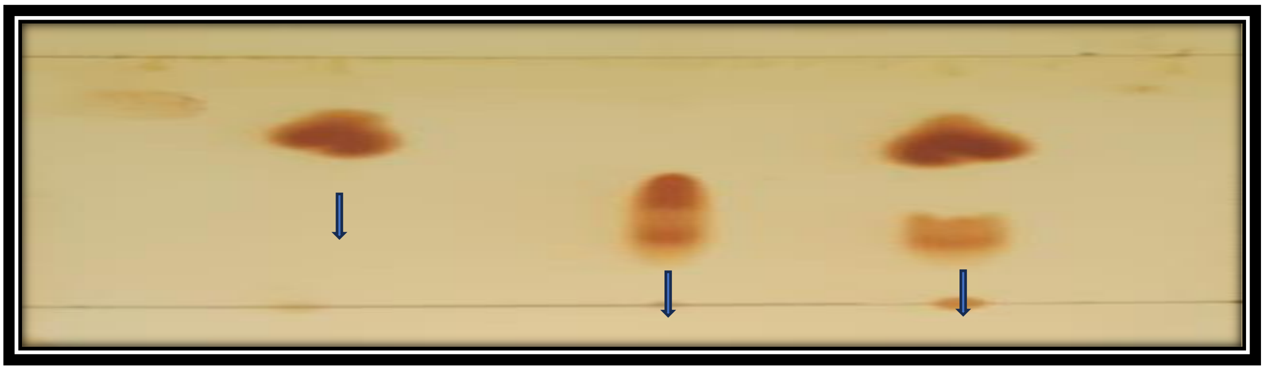


Figure 11 - Photograph of actual chromatogram showing separation of Paracetamol and Tramadol using mobile phase XIII

The Rf values of standard P, standard T and P+T pharmaceutical formulations in different mobile phases are shown in Table -1. By the study of table -1, it was found that the Rf values of P and T present in pharmaceutical formulation in combined form was found some different than Rf of std P and std T sample due to interaction of both drugs in combined form. In mobile phase XVI (only water), the TLC plate was developed for 20 minutes only and the movement of solvent take place till 4.2 cm. The movement of P take place till 3.7 cm and its Rf was calculated 0.88 but movement of T was found very less that did not separate from the base line. It is showing that Tramadol is more polar than paracetamol so its interaction is more with stationary phase(silica)

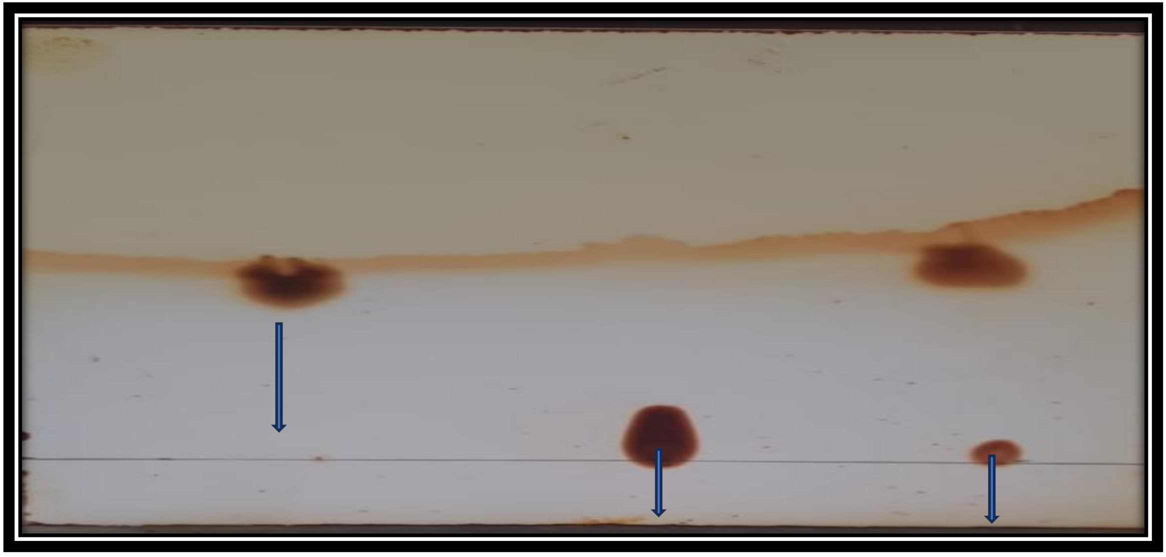


Figure 12 - Photograph of actual chromatogram showing separation of Paracetamol and Tramadol using mobile phase XVI

Identification of separated spots of combination tablet - Five combination tablets of Paracetamol and Tramadol were powdered. The powder was shaken with HPLC methanol(30ml) in a 100ml beaker. The solution was filtered. The residue left in the beaker, was again extracted with HPLC methanol and filtered. The combined filtrate was left at room temperature and concentrated solution was prepared. This solution was fed on TLC plate (20x20 cm^2). The plate was developed in successful solvent system (solvent system XII) figure – 9.

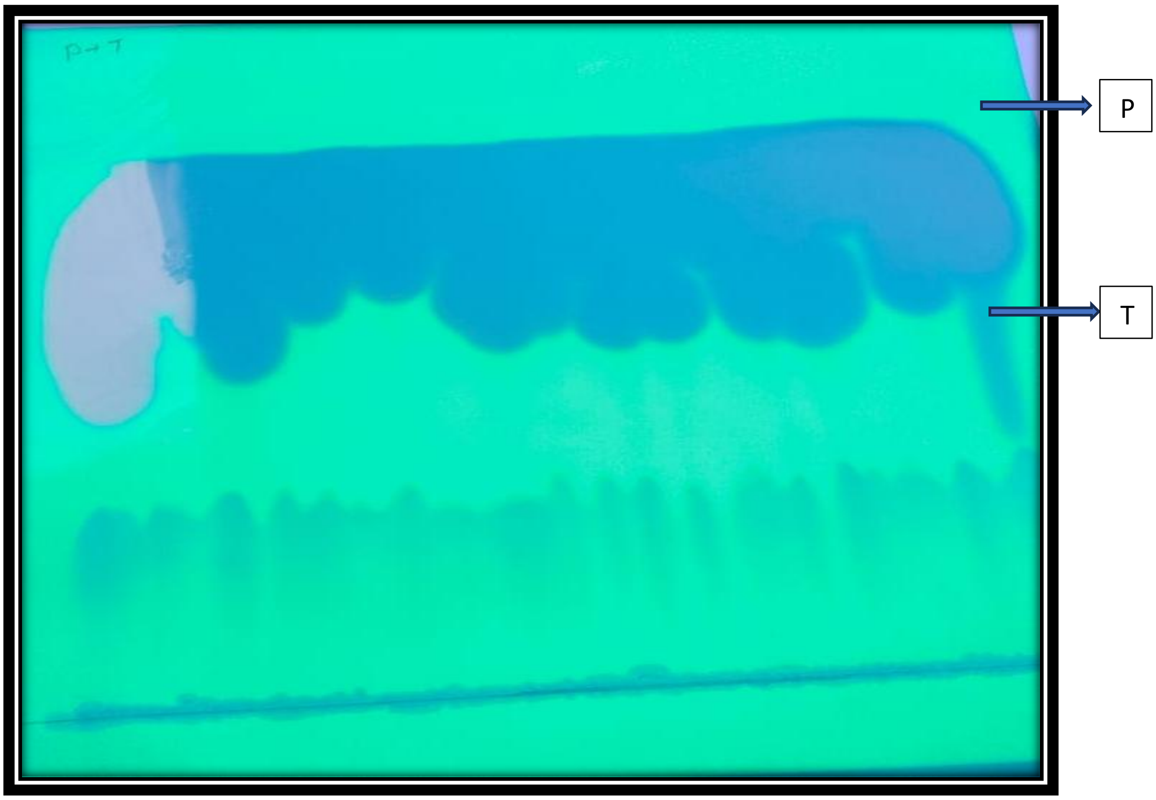
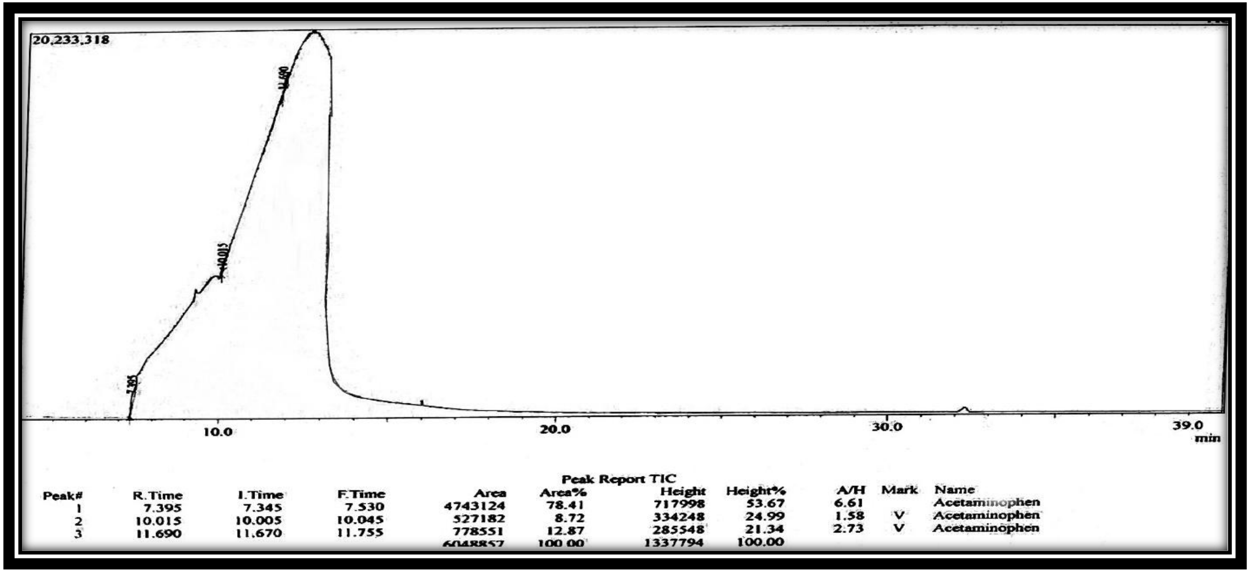


Figure 13- Photograph of actual chromatogram showing separation of Paracetamol and Tramadol using mobile phase XII

The separated bands as visible in figure - 9 were scratched and collected in separate beaker. The upper band was of Paracetamol as its Rf was similar to the control Paracetamol sample and the lower band was of Tramadol as its Rf was similar to the control Tramadol sample. Each scratched material was shaken with HPLC methanol(30ml) in a 100ml beaker. The solution was filtered. The residue left in the beaker, was again extracted with HPLC methanol and filtered. The crystals were dried separately. And mass spectra were recorded of both, separately.

The spectra of upper band were found similar to Paracetamol and the spectra of lower band were found similar to Tramadol. By instrumentation of separated bands, it was proved that separated bands were same to control samples. The GC-MS spectra of both scratched bands were pasted further.



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2	10.015	10.005	10.045	527182	8.72	334248	24.99	1.58	V	Acetaminophen
3	11.690	11.670	11.755	778551	12.87	285548	21.34	2.73	V	Acetaminophen
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Figure – 14 Gas Chromatogram of Scratched upper band

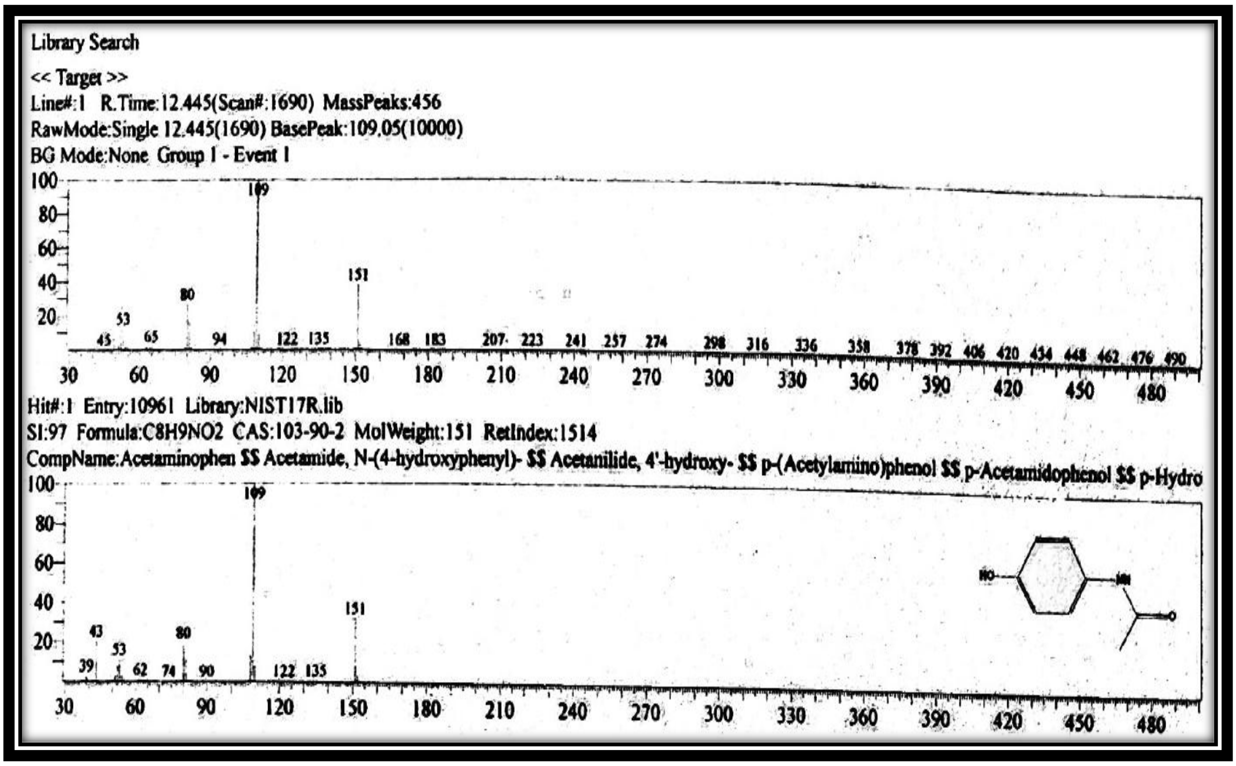

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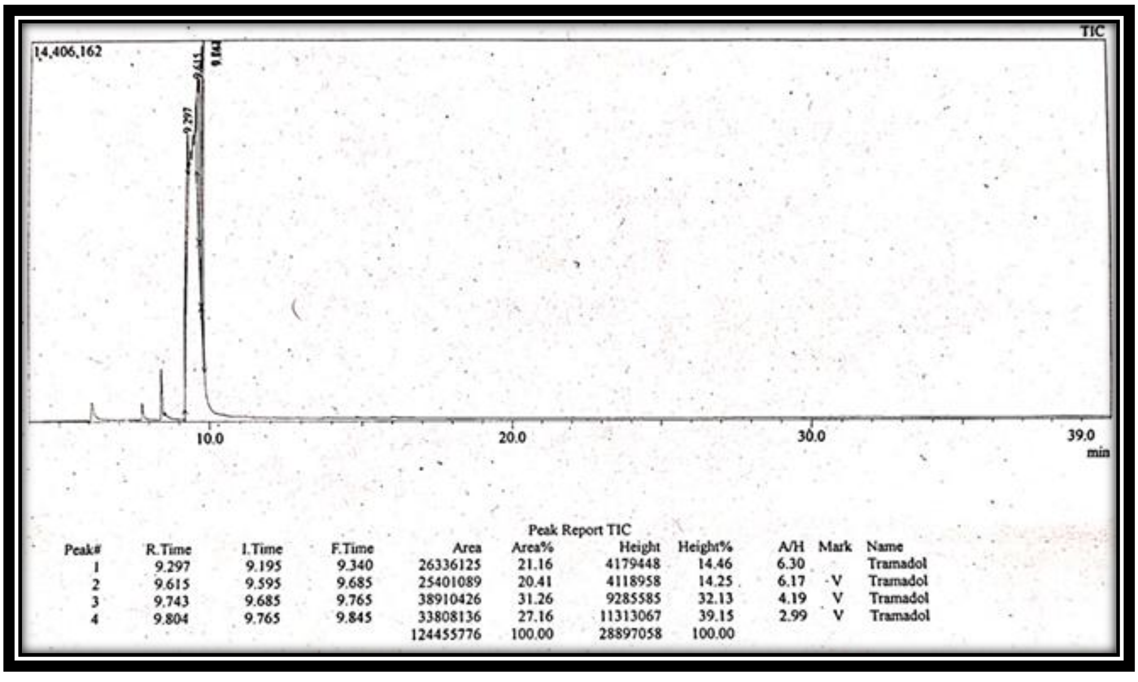
Figure – 15 Electron Ionization (EI) mass spectrum of scratched upper band

GC MS Spectra of scratched lower band -



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2	9.615	9.595	9.685	25401089	20.41	4118958	14.25	6.17	V	Tramadol
3	9.743	9.685	9.765	38910426	31.26	9285585	32.13	4.19	V	Tramadol
4	9.804	9.765	9.845	33808136	27.16	11313067	39.15	2.99	V	Tramadol
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Figure – 16 Gas Chromatogram of scratched lower band

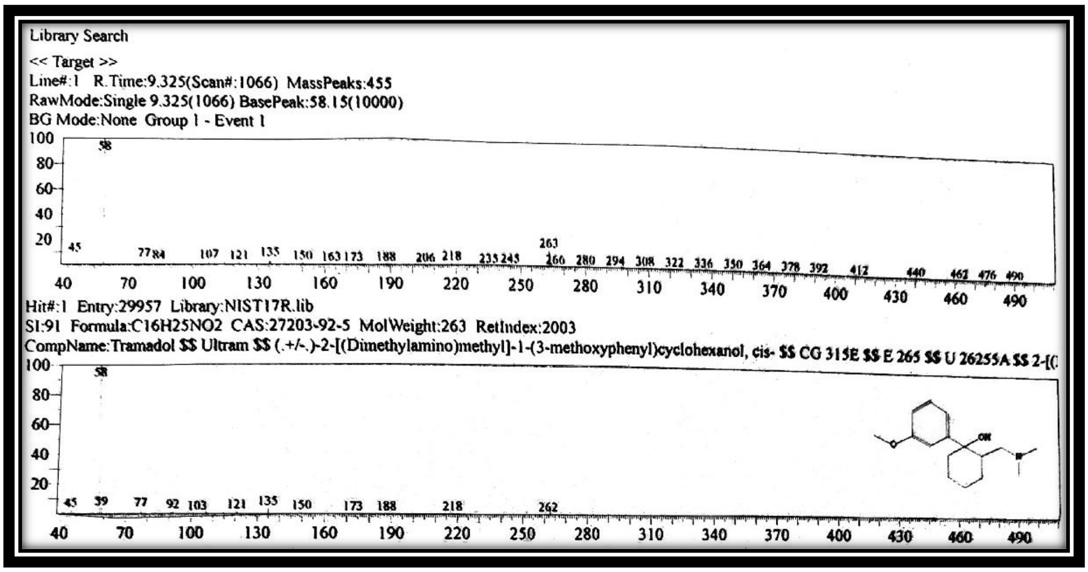

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Figure – 17 Electron Ionization (EI) mass spectrum of scratched lower band

CONCLUSION

Thin layer chromatographic technique is rapid inexpensive method for the separation of active components in pharmaceutical formulations. In studied 45 sustainable solvent systems, the 38 solvent systems were successful for the separation of both drugs (Graph -1) but in them only ten solvent systems - (1) Ethyl Acetate : Methanol :: 9:1 (v/v) (2)Ethyl Acetate : Methanol :: 8:2 (v/v) (3) Ethyl Acetate : Methanol: Acetic acid :: 16:4:1 (v/v) (4) Methanol: Toluene :: 19:1 (v/v) (5) Methanol: Toluene :: 18:2 (v/v) (6) Methanol: Toluene :: 17:3 (v/v) (7) Methanol: Toluene :: 16:4 (v/v) (8) Methanol: Toluene :: 14:4 (v/v) (9) Methanol: Toluene :: 7: 13(v/v) (10) Methanol: Toluene ::6:14 (v/v) have been found the best solvent systems to separate P and T pharmaceutical formulations because only in these systems compact spots of both drugs were obtaining. The universal and non- destructive method of visualisation using Iodine fuming was found to be very good for subsequent instrumental analysis for the eluted active components. Through such visions, we are paving the path for a more sustainable and eco-friendly approach to chemistry.

Conflict of interest – None to declare

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