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Prospective Use of Propylene Carbonate as a Mobile Phase Component in RP-HPLC

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ABSTRACT

The main objective of this article is to introduce propylene carbonate (PC) as a mobile phase component in HPLC. It is observed that PC and acetonitrile (ACN) have certain similarities in their chemical properties w.r.t. its use as an ideal organic mobile phase solvent. Hence it is speculated that if ACN is replaced by PC in already optimized RP-HPLC methods, no drastic changes would be observed in resolution, selectivity and efficiency. This was confirmed by comparing RP-HPLC methods developed for the separation and estimation of i) Fluconazole ii) Paracetamol and Tramadol, iii) Pioglitazone and Glimepiride iv) Oxcarbazepine and its impurities v) Chlorpyrifos with Isoproturon as internal standard, using both ACN and then PC as mobile phase component for each combination. All the developed and validated methods using PC have been found to meet the acceptance criteria as per ICH guidelines. This proves that PC is a good replacement for ACN. Moreover, unlike ACN, PC is an environmentally benign solvent. It is also cheaper as compared to ACN. Hence, implementation of this proposal would make future HPLC procedures both more economic and ecofriendly.

KEYWORDS: Organic modifier, propylene carbonate, acetonitrile, selectivity

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INTRODUCTION

High performance liquid chromatography (HPLC) is a diagnostic tool extensively used for identification and purification of organic or inorganic solutes in any biological, pharmaceutical, food, industrial, and environmental samples. Pharmaceutical and biotechnology industries use HPLC as it has applications in all phases of drug discovery, development and quality control.

While recognizing the merits of this technique, one has to remember that HPLC generates a large amount of organic and aqueous toxic solvent waste. HPLC use a column with typical internal diameter of 4.6 mm with most analytical methods using a flow rate of 1 mL/min. If a typical HPLC is used 20 hours per week, a single conventional HPLC would require 60L of solvent to operate per year. In other words, 60L of mixed organic/aqueous waste would be generated per year by a single HPLC instrument. This translates into 8 million liters of solvent produced per year to supply mobile phase for more than 1.5 lakh HPLC instruments in use today, while producing the equivalent of 40,000 55-gallon drums of waste per year. This waste mainly consists of organic modifiers like methanol (MeOH), acetonitrile (ACN), isopropanol and aqueous buffers all mixed together with low concentrations of analytes[1].

This waste cannot be directly disposed into the waste water streams because of the environmentally toxic organic solvents present in it. Hence, even though HPLC has become one of the most popular analytical techniques, HPLC waste disposal has always been a cause of major concern to chromatographers.

The most common options available to chromatographers to tackle this problem are, either, 1) to reduce the amount of solvents consumed or 2) to recover and reuse solvents.

Solvent consumption can be reduced by a) Transferring methods to smaller ID columns eg. UPLC or b) Shortening column lengths along with sorbent particle size. However, to implement these options, laboratories would be required to invest heavily in acquiring new columns and instruments.

Another emerging trend being considered by many analysts is to convert LC methods to SFC (Supercritical Fluid Chromatography). This would help to eliminate the problem of solvent waste disposal almost completely. However, the mobile phase chemistry in SFC is very different from conventional analytical LC. Hence, existing HPLC methods would have to be regenerated for SFC, requiring time and effort without a guarantee of success.

The next option is the recovery and reuse of the solvents from HPLC solvent waste. For this purpose, laboratories and production plants must be equipped with automated solvent recovery units that enable collection of used solvent, distillation of solvent mixtures, gas chromatography control of the quality

and composition, readjustment of composition by adding one solvent (if needed), and recycling the solvent into the HPLC process[2]. However, very few pharmaceutical manufacturers or laboratories generate sufficiently large volumes of solvent waste to warrant recovery themselves.

Hence, to make solvent recovery commercially viable, pooling of solvent wastes by different organizations is required so that the problem of inadequate starting volume does not remain an issue.

Thus chromatographic scientists are in search of newer and better methods which would make HPLC solvent waste disposal economically feasible.

In this article we suggest a solution for the above discussed problem. We propose to replace ACN, a commonly used solvent in HPLC, with Propylene Carbonate (PC). Till date, PC has been mainly used as a reactive intermediate or as an inert solvent in various industries. It is used in degreasing, paint stripping and cleaning applications. It is also used as a carrier solvent for topically applied medications and cosmetics. PC is widely employed as an organic solvent in electrolytes for high-energy density batteries. Other applications include its use in extraction of metals, hydraulic brake fluids, bleaching of wood etc. [3]

PC has never been used as a mobile phase component in HPLC. PC is an easily biodegradable solvent with a very high value of LD_{50}. Hence, if this proposition is implemented commercially, the problem of HPLC solvent waste disposal would be solved to a great extent.

ACN forms an azeotropic mixture with water and MeOH which are often present in HPLC solvent wastes, making straightforward distillation unlikely to achieve the required purity levels (99.95 %). Instead, additional separation techniques are needed, possibly including 'washing' the wash stream with another solvent in order to extract ACN before further processing this by fractional distillation[4].

If a manufacturer cannot recycle the solvent waste containing ACN, then arrangements should be made for the collection and transport of these highly inflammable toxic wastes to large incinerators where they can be incinerated. However, not only is this an additional financial burden for the manufacturers, but also an energy intensive process which contributes to green house gas emissions. Additionally, when incinerating ACN containing solvent mixtures, special incinerators are required, as nitrogen oxide fumes that would be released subsequently are harmful to the environment. Manufacturers should also take care that these emissions are within the acceptable limits set by EPA and other such government organizations.

PC has several advantages over ACN, a few of which are listed below:

a) Higher boiling point and flashpoint temperature: This would reduce the chances of accidental fires in laboratories.

b) Higher values of dipole moment and dielectric constant: This would help in better chromatographic separation.

c) Higher biodegradability and lower capacity to bioaccumulate: Therefore, PC is more environments friendly.

The properties of PC and ACN are compared in Table 1.

Table1: Comparative properties of PC and CAN

Sr. No.	Property	PC	ACN
1	Molecular Formula	$C_4H_6O_3$	C_2H_3N
2	Molecular Weight	102.1	41
3	Boiling point (°C)	242	81.60
4	Freezing/Melting point (°C)	-55	-43.8
5	Specific gravity	1.2059	0.78
6	Enthalpy of Vapourisation KJ/mol.	31.55	8.33
7	Refractive index	1.42	1.34
8	Specific Heat cal/gm	2.50[a]	0.38
9	Dipole moment 20°C debyes	5.00	3.44
10	Dielectric constant e.s.u	64.4	37.5
11	Flash point temperature (°C)	135.0	5.0
12	Polarity Index (P)	6.1	5.8
13	Acute toxicity LD_{50} (on rats mg/kg)	29100	2460
14	Vapour Pressure mpa	0.03	97
15	Viscosity (cp)	2.5	0.38
16	Log $P_{o/w}$	-0.41	-0.34
17	Partition coefficient	0.3890	0.4036

In spite of the above mentioned advantages, PC has two major drawbacks which can prevent its use as a mobile phase component. They are its 1) low miscibility with other solvents2) high viscosity.

EXPERIMENT

PC is miscible with methanol along with six other solvents. Among various proportions experimented with, it was found that PC: MeOH:: 60:40 (v/v) showed complete miscibility with water as well as other organic solvents under all conditions. It also showed minimum molar volume and acceptable viscosity. Hence a mixture of PC and MeOH in the ratio of 60:40, further referred to as Solvent-X, was used for HPLC method development and validation.

Table 2: Comparison of properties of Solvent-X and ACN

Property	Solvent-X	ACN
UV Cut off (nm)	210	190
Boiling Point (°C)	Non azeotropic mixture	81.60
Viscosity (mPa.s)	1.009	0.343
Partition coefficient	0.242	0.4036
Specific Gravity	1.042	0.79

Two separate RP-HPLC methods were developed and validate during ACN and Solvent-X respectively for the simultaneous separation and estimation of the following:-

1. Fluconazole and Satranidazole
2. Paracetamol and Tramadol HCl
3. Pioglitazone and Glimepiride
4. Oxcarbazepine and its impurities
5. Chlorpyrifos with an internal standard

For all the 5 applications, flow rate was 1mL/min, Column Temperature was ambient and Injection volume was 5µL.

RESULTS AND DISCUSSION

The optimized chromatographic conditions are listed in Table 2, 4, 6, 8 and 10.

Developed methods were also successfully applied for the separation of drugs, impurities and pesticides in commercially marketed products.

ESTIMATION OF FLUCONAZOLE WITH AN INTERNAL STANDARD METHOD

Table 3: Optimized Chromatographic conditions for the estimation of Fluconazole

	Method A (Solvent-X)	Method B (Acetonitrile)
Equipment Used:	JASCO HPLC 1500 series	JASCO HPLC 1500 series
Mobile Phase:	Solvent-X : Water (60:40, v/v)	Acetonitrile: Water (60:40, v/v)
Column:	Kromasil 100-5 C18 (250 x 4.6 mm, 5 µ)	Kromasil 100-5 C18 (250 x 4.6 mm, 5 µ)
Detector:	UV Wavelength (220nm)	UV Wavelength (220nm)
Retention Time:	Fluconazole – 4.70mins Satranidazole – 6.97mins	Fluconazole – 2.69mins Satranidazole – 3.66mins
Run Time:	8min	7min

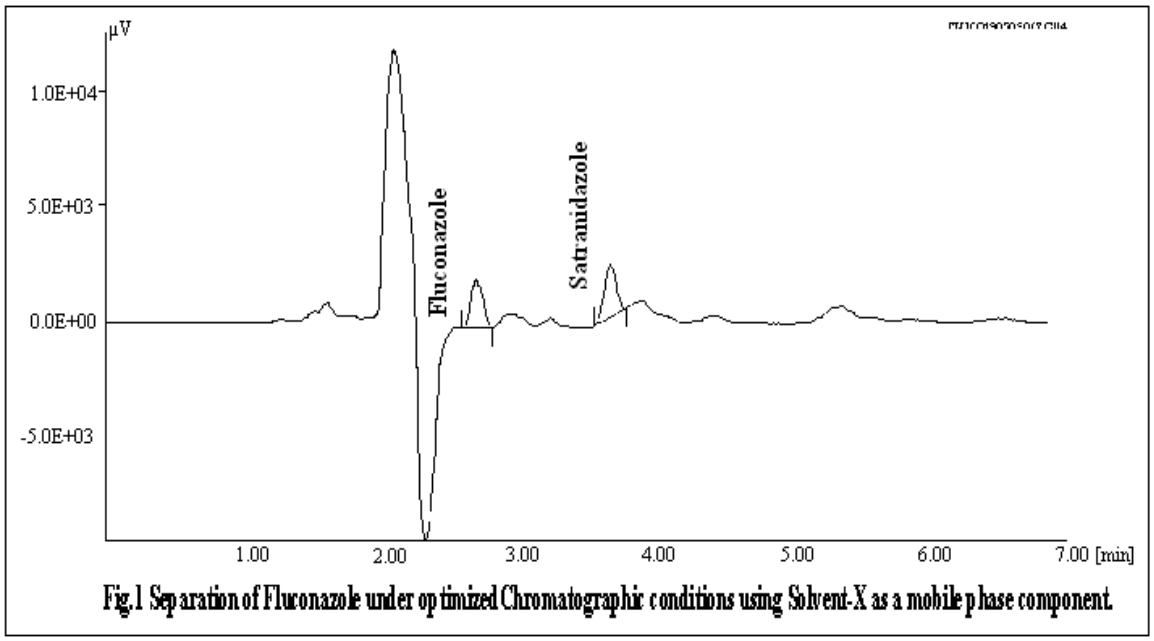

Fig.1 Separation of Fluconazole under optimized Chromatographic conditions using Solvent-X as a mobile phase component.

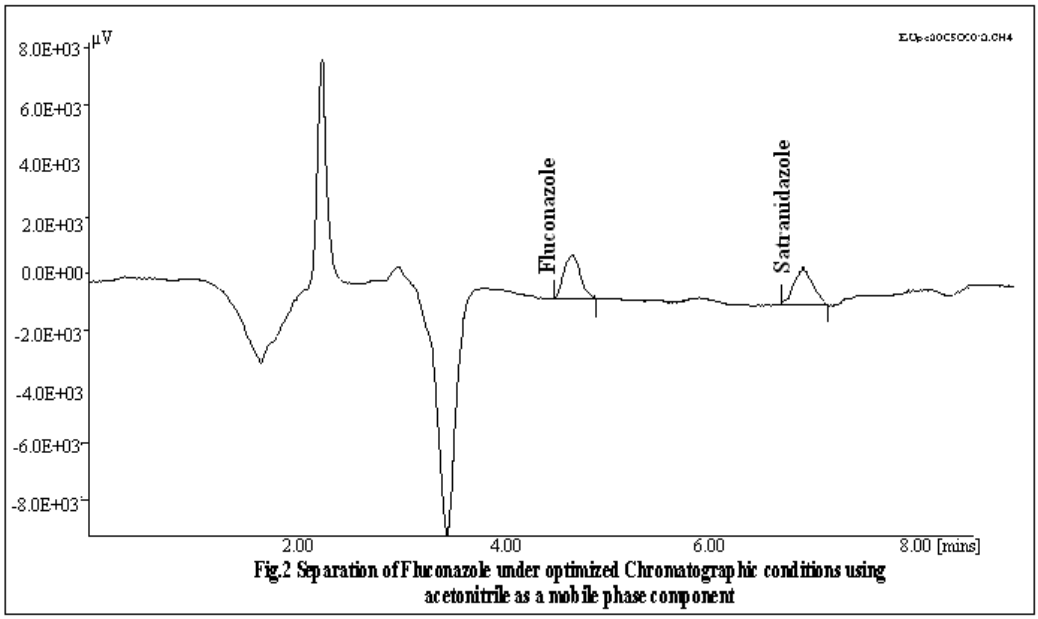

Fig.2 Separation of Fluconazole under optimized Chromatographic conditions using acetonitrile as a mobile phase component

Table 4: Chromatographic figures of merit for Fluconazole and Satranidazole

Parameter	Method (A)		Method (B)	
	Fluconazole	Satranidazole	Fluconazole	Satranidazole
Retention Time (R_t)	4.71	6.97	2.69	3.66
Retention Factor (α)	1.07	2.07	0.28	0.78
Resolution (Rs)	-	5.27	-	4.00
Peak Symmetry (A_s)	1.25	1.00	1.1	1.25
Theoretical Plates (N)	15412	21563	22414	32631

ESTIMATION OF PARACETAMOL AND TRAMADOL

TABLE 5: Optimized chromatographic conditions for the separation of Paracetamol and Tramadol

	Method A (Solvent-X)	Method B (Acetonitrile)
Equipment Used:	JASCO HPLC 900 series	JASCO HPLC 900 series
Mobile Phase:	Solvent-X : Buffer (0.1M Tetra butyl Ammonium Hydrogen Sulfate)::10:90, v/v	Acetonitrile : Buffer (0.1M Tetra butyl Ammonium Hydrogen Sulfate)::10:90, v/v
Column:	Inertsil ODS-3 (250x4.6 mm)5µm	Inertsil ODS-3 (250x4.6 mm)5µm
Detector:	UV-Vis (276nm)	UV-Vis (276nm)
Retention Time:	Tramadol – 3.26 min Paracetamol – 4.16 min	Tramadol – 3.49 min Paracetamol – 3.64 min
Run Time:	6 min	6 min

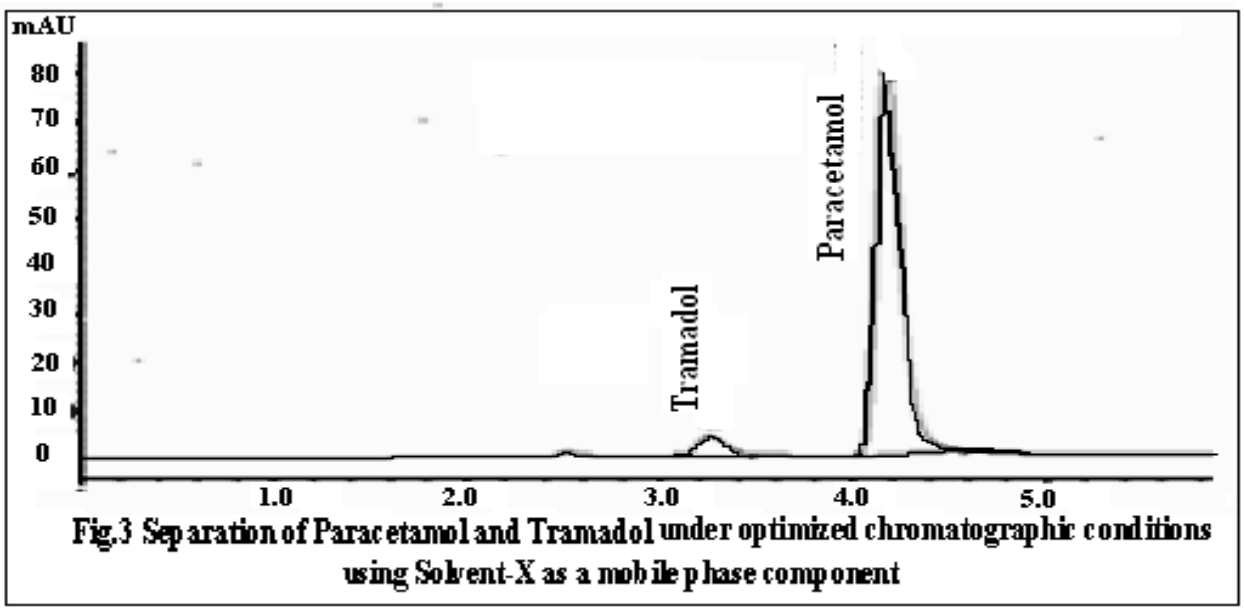

Fig.3 Separation of Paracetamol and Tramadol under optimized chromatographic conditions using Solvent-X as a mobile phase component

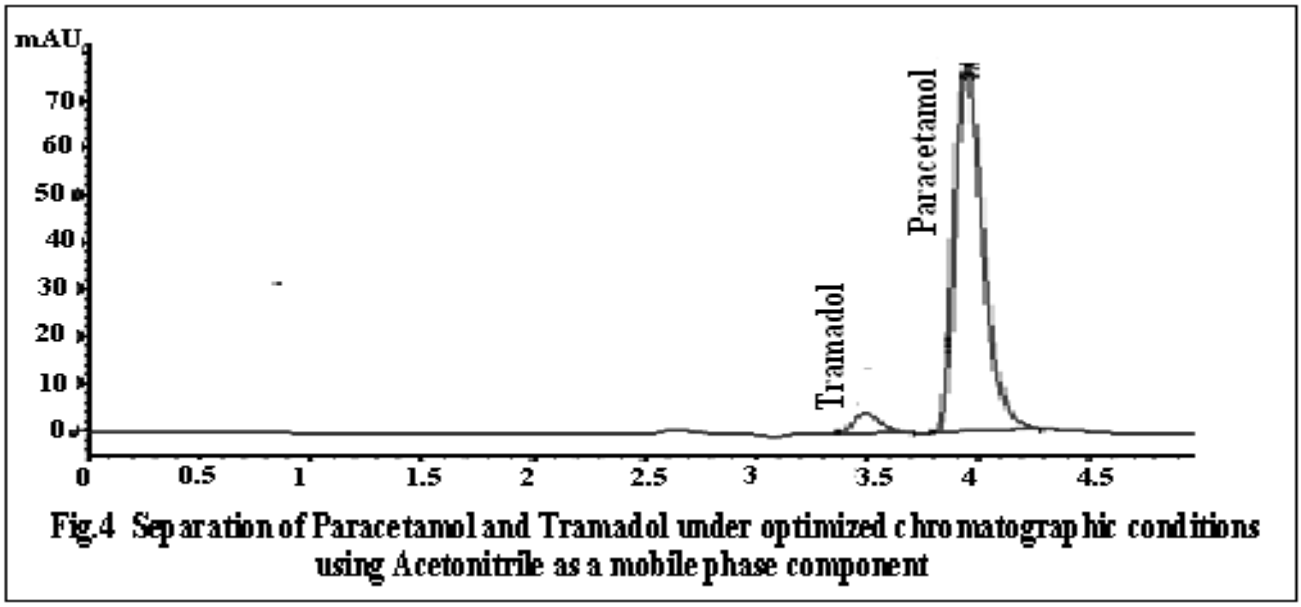

Fig.4 Separation of Paracetamol and Tramadol under optimized chromatographic conditions using Acetonitrile as a mobile phase component

TABLE 6: Chromatographic figures of merit for Paracetamol and Tramadol HCl

Parameter	Method (A)		Method (B)	
	Tramadol	Paracetamol	Tramadol	Paracetamol
Retention Time (R_t)	3.26	4.16	3.49	3.64
Retention Factor (α)	0.25	0.52	0.31	0.48
Resolution (Rs)	-	1.56	-	1.66
Peak Symmetry (A_s)	1.1	1.1	1.0	1.1
Theoretical Plates (N)	15078	18492	35899	45592

ESTIMATION OF PIOGLITAZONE AND GLIMEPIRIDE

TABLE 7: Optimized chromatographic conditions for the separation of Pioglitazone and Glimepiride

	Method A (Solvent-X)	**Method B (Acetonitrile)**
Equipment Used:	JASCO HPLC 900 series	JASCO HPLC 900 series
Mobile Phase:	Solvent-X : Water :: 60 : 40 + 10 µL Glacial acetic acid	Acetonitrile : Water :: 60 : 40 + 10 µL Glacial acetic acid
Column:	Agilent Cyano (250 mm x 4.6 mm, 5 µm)	Agilent Cyano (250 mm x 4.6 mm, 5 µm)
Detector:	UV Wavelength (230nm)	UV Wavelength (230nm)
Retention Time:	Pioglitazone – 3.8mins Glimepiride – 6.3mins	Pioglitazone – 5.7mins Glimepiride – 7.8mins
Run Time:	8.0min	10.0min

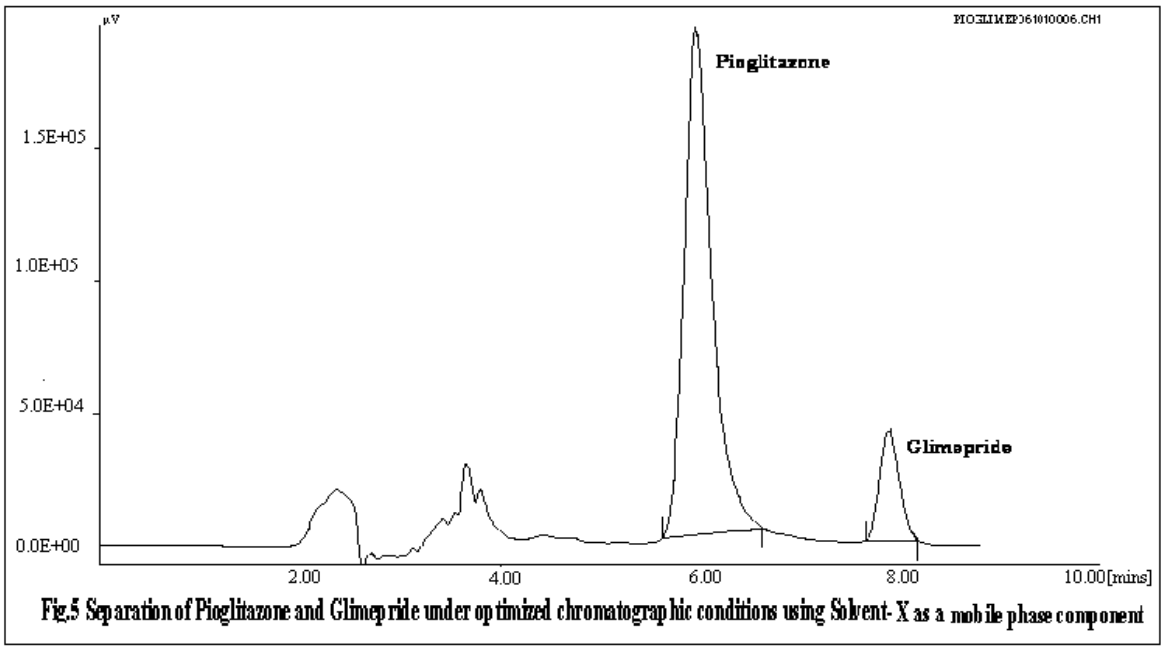

Fig.5 Separation of Pioglitazone and Glimepride under optimized chromatographic conditions using Solvent-X as a mobile phase component

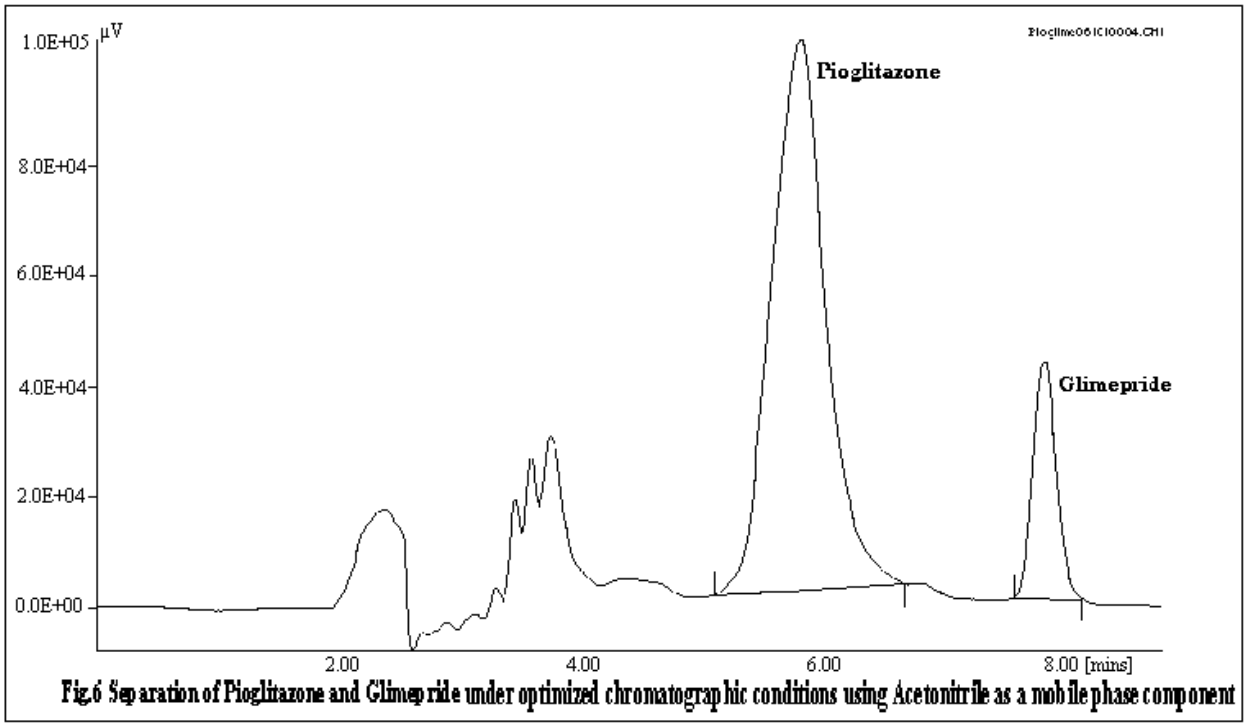

Fig.6 Separation of Pioglitazone and Glimepride under optimized chromatographic conditions using Acetonitrile as a mobile phase component

TABLE 8: Chromatographic figures of merit for Pioglitazone and Glimepiride

Parameter	**Method (A)**		**Method (B)**	
	Pioglitazone	Glimepiride	Pioglitazone	Glimepiride
Retention Time (R_t)	3.8	6.3	5.7	7.8
Retention Factor (α)	0.775	1.95	1.52	2.35
Resolution (Rs)	-	5.87	-	2.3
Peak Symmetry (A_s)	1.0	1.0	1.0	1.0
Theoretical Plates (N)	1260	3480	3249	4622
LOQ (ng/mL)	400	100	300	40

ESTIMATION OF OXCARBAZEPINE AND ITS IMPURITIES

TABLE 9: Optimized chromatographic conditions for the separation of Oxcarbazepine and its impurities

	Method A (Solvent-X)	Method B (Acetonitrile)
Equipment Used:	AGILENT HPLC 1100 series	AGILENT HPLC 1100 series
Mobile Phase:	Solvent-X: 10mMAmmonium Acetate::40:60, pH 3.0 with dil. Acetic Acid	ACN: 10mMAmmonium Acetate::40:60, pH 3.0 with dil. Acetic Acid
Column:	Symmetry C_{18}, 250mm x 4.6mm, 5µm	Symmetry C_{18}, 250mm x 4.6mm, 5µm
Detector:	PDA,UV-Vis (239nm)	PDA,UV-Vis (239nm)
Retention Time:	Oxcarbazepine - 5.6mins Impurity 1 - 8.0mins Impurity 2 - 8.8mins	Oxcarbazepine - 5.8mins Impurity 1 - 8.6mins Impurity 2 - 9.3mins
Run Time:	10min	10min

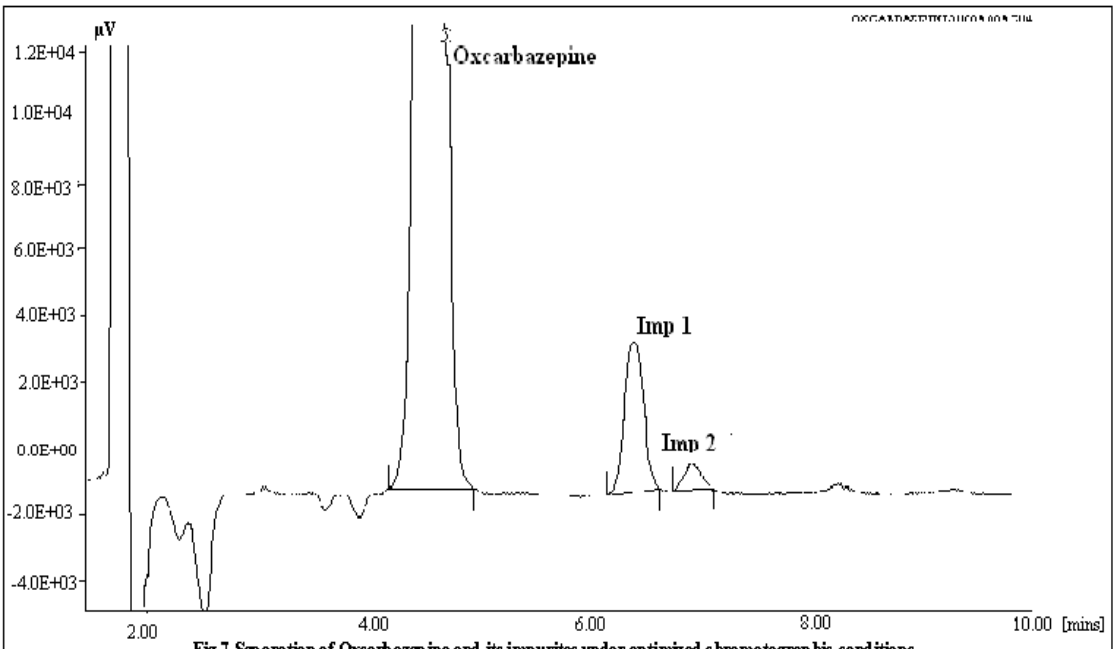

Fig.7 Separation of Oxcarbazepine and its impurites under optimized chromatographic conditions using Solvent-X as a mobile phase component

TABLE 10: Chromatographic figures of merit for Oxcarbazepine and its impurities

Parameter	Method (A)			Method (B)		
	Oxcarbazepine	Impurity 1	Impurity 2	Oxcarbazepine	Impurity 1	Impurity 2
Retention Time (R_t)	5.6	8.0	8.8	5.808	8.642	9.358
Retention Factor (α)	1.367	2.23	2.63	0.90	1.79	2.019
Resolution (Rs)	-	5.8	2.1	-	11.15	2.86
Peak Symmetry (A_s)	1.23	1.22	1.17	1.17	1.10	1.13
Theoretical Plates (N)	6539	8290	8899	12499	16091	22368
LOQ(ng/mL)	250	225	500	250	225	500

ESTIMATION OF CHLORPYRIFOS AN INTERNAL STANDARD METHOD

Table 11: Optimized chromatographic conditions for the separation of Chlorpyrifos

	Method A (Solvent-X)	Method B (Acetonitrile)
Equipment Used:	JASCO HPLC 900 series	JASCO HPLC 900 series
Mobile Phase:	Solvent-X : Water :: 90:10	Acetonitrile : Water :: 90:10
Column:	Inertsil ODS-3 (250 x 4.6mm), 5µm	Inertsil ODS-3 (250 x 4.6mm), 5µm
Detector:	UV-Vis (230nm)	UV-Vis (230nm)
Retention Time:	Isoproturon – 3.8mins Chlorpyrifos – 8.3mins	Isoproturon – 3.6mins Chlorpyrifos – 6.8mins
Run Time:	10.0min	8.0min

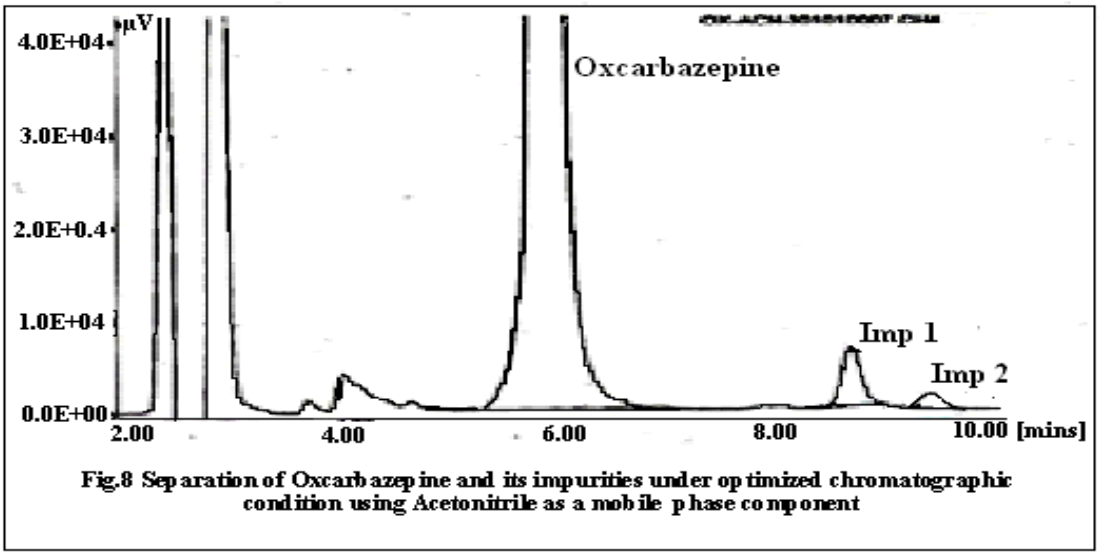

Fig.8 Separation of Oxcarbazepine and its impurities under optimized chromatographic condition using Acetonitrile as a mobile phase component

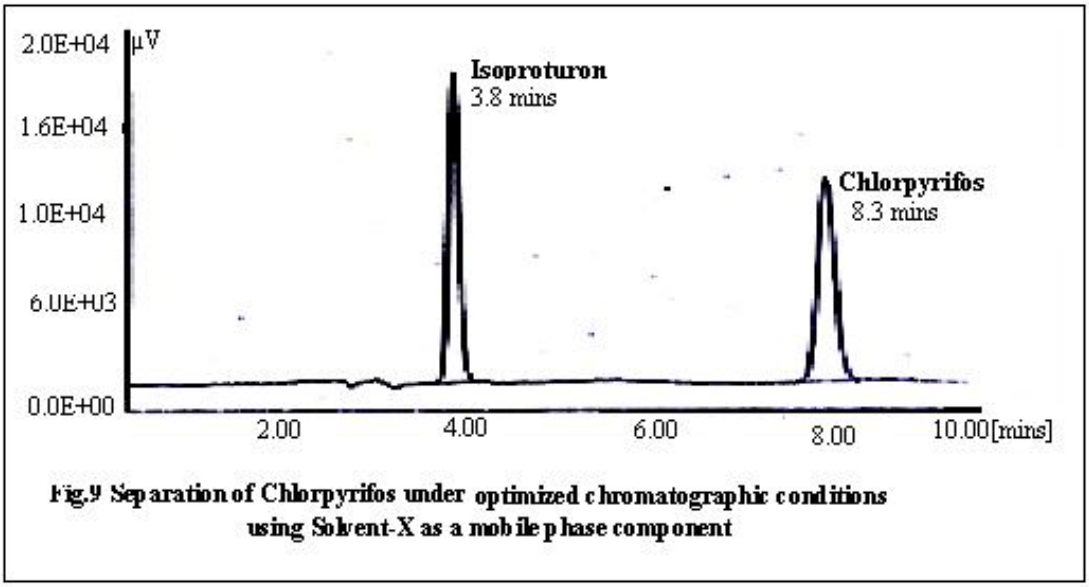

Fig.9 Separation of Chlorpyrifos under optimized chromatographic conditions using Solvent-X as a mobile phase component

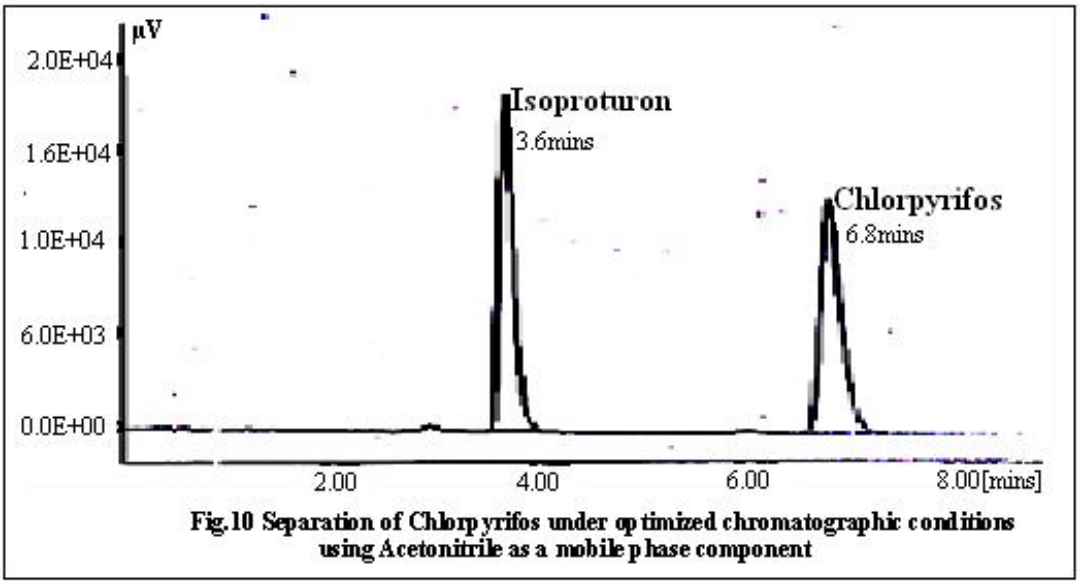

Fig.10 Separation of Chlorpyrifos under optimized chromatographic conditions using Acetonitrile as a mobile phase component

TABLE 12: Chromatographic figures of merit for Isoproturon and Chlorpyrifos

Parameter	Method (A)		Method (B)	
	Isoproturon	Chlorpyrifos	Isoproturon	Chlorpyrifos
Retention Time (R_t)	3.8	8.3	3.6	6.8
Retention Factor (α)	0.33	1.72	0.30	1.36
Resolution (Rs)	-	7.41	-	5.0
Peak Symmetry (A_s)	1.36	1.22	1.42	1.35
Theoretical Plates (N)	3957	4304	3632	5975
LOQ (ng/mL)	150	300	150	300

All the above five methods have been completely validated in accordance with ICH guidelines. Thus, it is confirmed that rapid, precise, accurate, robust and rugged RP-HPLC methods can be developed using Solvent-X instead of ACN as a mobile phase constituent. When the methods developed using ACN are compared with those developed using Solvent-X, it is seen that PC shows selectivity and efficiency similar to ACN. Also, since no undesired interferences have been observed in the HPLC methods, it is proved that Solvent-X is compatible with other organic solvents and buffers used in RP-HPLC. Thus, there is a lot of scope for research in this field which would help in contributing to reducing the amount of toxic waste generated through HPLC analysis.

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