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Analysis of Alkyl, Aryl Sulphonate Ester – Genotoxic Impurities (GIS) In Active Pharmaceutical Ingredient (API's) and drug product

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ABSTRACT

This is latest series of review focused on the analysis of the genotoxic impurities (GIs),with specific reference to alkyl, aryl sulphonateester. Such reactive materials are commonly used as raw materials, reagents and intermediates in the chemical synthesis of new drugs in pharmaceutical research and development. This article reports the latest developments in the limit for controlling sulfonate esters in drug formulation doses by various regulatory environment and the latest developments in analysis culminating in a review of analytical approaches in literature. The literature is sub-categorized by technique of separation (gas chromatography (GC), high-performance liquid chromatography (HPLC) and further tabulated by type of Active Pharmaceutical Ingredient (API) and impurity with brief information and references of the process. Such a wide range of options allow the analyst to choose the most suitable technique specific to their needs.

Keywords: Alkyl and Aryl Sulphonate Ester, Genotoxic Impurity, Chromatographic, Spectroscopic, Derivatization

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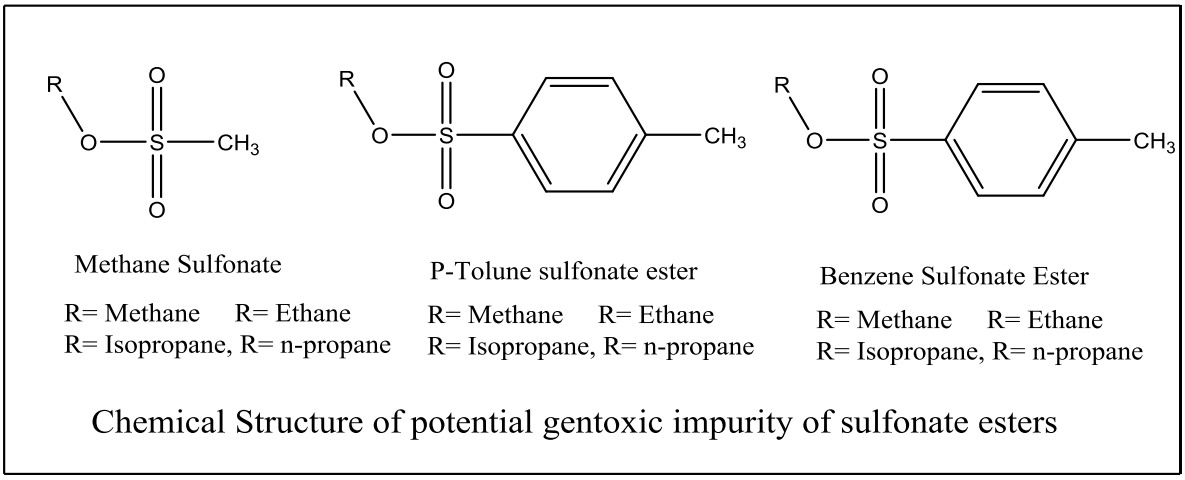
INTRODUCTION

The issue of Genotoxic Impurities (GIs) received an attention from recent past year focus on ester of alkyl and aryl sulfonic acids having genotoxic impurities, Sulphonate moiety is readily displaced by a variety of nucleophiles, such esters can act as DNA alkylating agents in biological systems and have been shown to exert genotoxic effects in bacterial and mammalian cells.[1] Sulfonic acids including methane sulfonates, benzene sulfonate, and p- toluene sulfonate are used for salt formation and acid catalysis during production of drug substance. [2] The common salt of drugs having alkyl and aryl sulfonic acid had Mesylate, Ethane sulfonate, Tosylate, Besylate, Triflate, Closylate, Pipsylate, Nosylate, Camsylate are common salt containing APIs. Exposure of alkylation reagent can result in the formation of number of DNA mutation that causes the molecular DNA damage and not repaired properly and they could lead a permanent mutation causing the genetic instability,and mutation during the replication cancer produce.[3] The generic structure of alkyl sulfonate ester was shown in below.



Chemical Structure of potential gentoxic impurity of sulfonate esters

Sulfonates are use in various associated reaction like API salt forming agent, cyclization, good leaving group, protecting group, double bond migration, esterification, enamine-amine reduction like reaction in synthesis of drug products sulfonates use.[4] Methane sulfonic acid, benzene sulfonic acid, p-Toluene sulfonic acid and sulfuric acid are commonly used for salt formation of API as reagent in synthesis of Methanol, ethanol, propanol or isopropanol used as solvent for purification and crystallization of drug substance, Interaction between sulfonic acid and alcohols leads to formation of alkyl sulfonate ester which having shown potent genotoxic effect.[5] The salts formation is the most useful approach to optimization of the physicochemical properties, Stability of APIs and Formulation. Each form of salts in particular API or formulation having different physicochemical property and Stability of drug.[6] Sulfonates are common salts to form addition advantages of produce higher melting points, increase solubility of APIs, increase stability of drug and also secondary

processing like granulation, higher melting points also beneficial for avoid caking and aggregation in Formulation.[7] In generic/compound -having specific limits for the alkyl or aryl sulfonate ester and chloroalkane can be the toxicological data and provision of the International Conference of Harmonization (ICH) M7 Guidelines.[8]

Alkylating compounds can vary greatly in their reactivity towards DNA. One property of an alkylating compound, which can determine DNA reactivity, is whether the nucleophilic attack occurs via an SN1 or SN2 mechanism. Compounds that react with DNA bases via an SN2 mechanism will primarily lead to N-alkylation because they are selective for highly nucleophilic sites. Whereas a compound able to react via an SN1 mechanism will lead to high levels of O-alkylation. Due to alkylating agent efficiency of DNA repair mechanisms and exposure dose can also influence the observed genotoxicity.[9]

Analytical approach to trace analysis of alkyl or aryl sulfonic acid containing APIs

Acceptable limits and general overview according to various regulatory agencies**:**

The European pharmacopeia has been firstly highlighting in these areas. The European Directorate for the Quality of Medicine (EDQM) firstly requested to the information and need of Pharmacopeia limit test for the alkyl or aryl sulfonate impurities in various APIs salts. The ICH Q9 guidelines are concerning the genotoxic impurities form in the saltformation of APIs, formation of these impurities needs for regularly testing according to various ICH guidelines[6]. The generic Chemical structures of potential genotoxic impurities of alkyl or sulfonate ester shown in fig no 1. The common salt of drugs having alkyl and aryl sulfonic acid had Mesylate, Ethane sulfonate, Tosylate, Besylate, Triflate, Closylate, Pipsylate, Nosylate, Camsylate are common salt containing APIs and formulation.

Limitof the genotoxic impurities which producesside effect according to various regulatory guidelines are given in below [3,10]

Table 1: ICH M7 Guideline- Treatment duration and Daily intake.

Duration of treatment	≤1 month	> 1-12 months	> 1-10 years	> 10 years to lifetime
Daily Intake (µg/day)	120	20	10	1.5

Table 2: ICH Q3A- Reporting, Identification and Qualification Threshold.

Maximum Daily Dose 1	**Reporting threshold 2**	**Identification threshold 3**	**Qualification threshold**
<2g/day	0.05%	0.10%or 1.0mg/day intake (whichever is lower)	0.15%or 1mg/day intake (whichever is lower)
>2g/day	0.03%	0.05%	0.05%

Table 3: EMEA guideline on duration of clinical exposure.

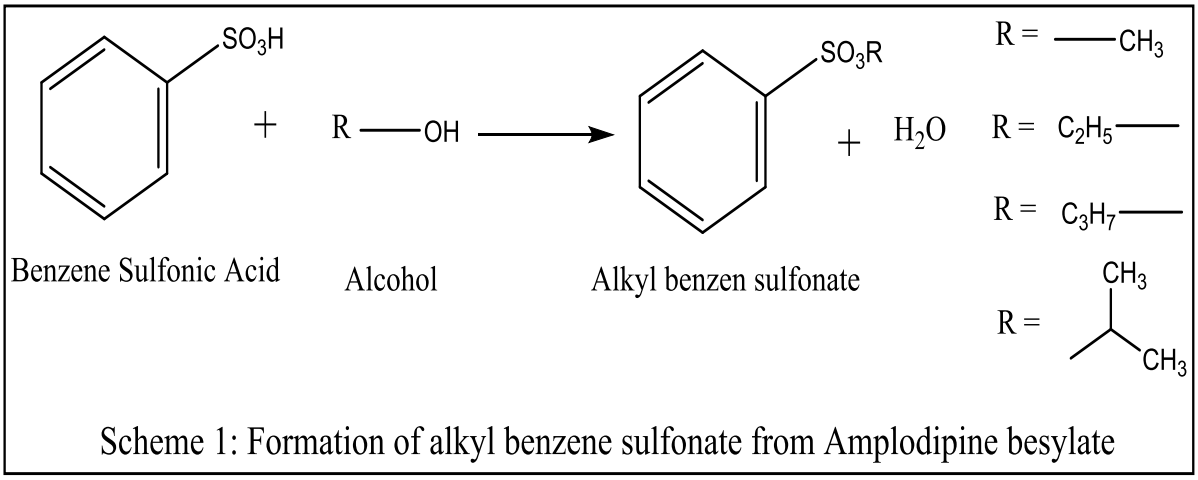
	Duration of clinical exposure					
	Single dose	**>single dose to <1 month**	**>1month to <3 months**	**>3month to <6month**	**>6month to <12 month**	**>12months or at marketing**
Staged TTC (g/day)	120	60	20	10	5	1.5

Table 4: FDA guideline on duration of exposure and impurity concentration limit based on drug daily dose.

Duration of exposure	**Allowable daily Intake**	**Impurity concentration limit (ppm) Based on drug daily dose**								
		1mg	**5mg**	**10mg**	**50mg**	**0.1g**	**0.2g**	**0.5g**	**1.0g**	**2.0g**
> 12 months	1.5µg/day	150	300	150	30	15	7.5	3	1.5	0.75
≤ 12 months	5 µg/day	5000	1000	500	100	50	25	10	5	2.5
≤ 6 months	10 µg/day	5000	2000	1000	200	100	50	20	10	5
≤ 3 months	20 µg/day	5000	4000	2000	400	200	100	40	20	10
≤ 1 months	60 µg/day	5000	5000	5000	1200	600	300	120	60	30
Single dose	120 µg/day	5000	5000	5000	2400	1200	600	240	120	60

High performance Liquid chromatography:

By using a HPLC Pharmacopeial method involving derivatization with alkyl benzenesulfonate (see scheme 1) which having potent genotoxicity in various drug salt. [12]

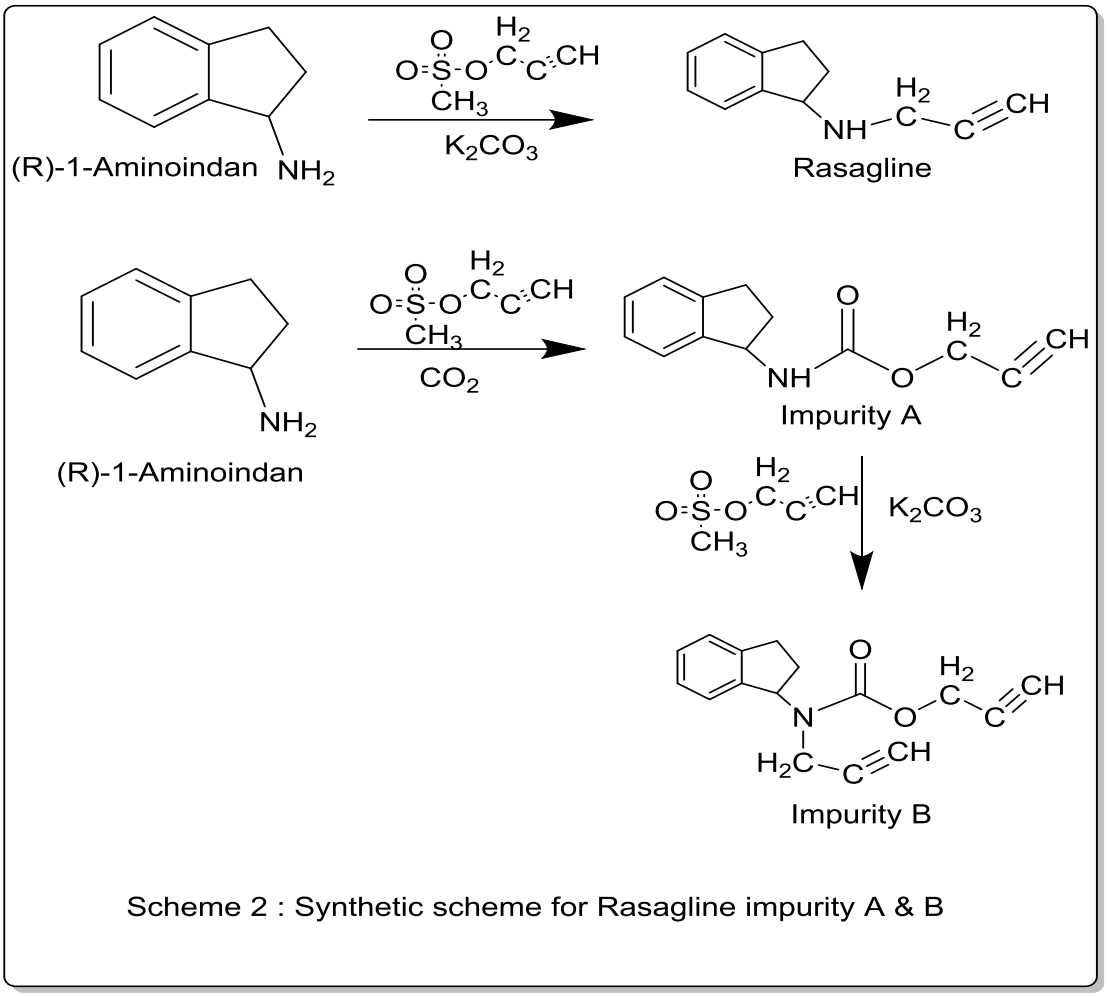


Scheme 1: Formation of alkyl benzene sulfonate from Amplodipine besylate

For the stability of API & Its formulation convert into Various salts form by using sulfonic acids like Mesylate, Ethane sulfonate, Tosylate, Besylate, Triflate, Closylate, Pipsylate, Nosylate, Camsylate. These sulfonic acid salts produce genotoxicity by alkylation of DNA.

The LOD 7,11,12& 9 ppm for Methyl Benzene Sulphonate, Ethyl Benzene Sulphonate, N-Butyl Benzene Sulphonate& Iso-Propyl Benzene Sulphonate impurity respectively in amlodipine besylate and LOQ was found to 31,32,35 & 28ppm respectively. The HPLC data will summarized to Table 1[12.] Nonvolatile GTIs are usually analyzed by high performance liquid chromatography (HPLC)

technique, where reversed phase (RP) HPLC is the most widely used separation mode compared with the normal one.A control strategy for genotoxic impurities is based on understanding the source and entry point of the impurity. In this article two genotoxic impurity N-(2-methyl-5-aminophenyl)-4-(3pyridyl)-2-pyrimidine amine (impurity-1) and 4(4-methyl piperazinyl methyl) benzoic acid dihydrochloride (impurity-2) present in Imatinib mesylate human carcinogen, its presence is limited to 10 ppm in the drug substance imatinib mesylate.[14] Lopinavir& Ritonavir taken as combination in the treatment of HIV protease inhibitor in Anti-HIV treatment but it contains Methyl methane sulfonate (MMS) & Ethyl methane sulfonate (EMS) containing potential genotoxic impurity having limit according to EMEA and US-FDA guideline having maximum daily dose of MMS and EMS 1.5 µg/day and maximum daily dose of Lopinavir and Ritonavir, MMS and EMS to be controlled at combined limit of 1.4 µg/day & 1.25 µg/day.LOD &LOQ of MMS & EMS 0.002 µg/ml & 0.01 µg/ml respectively. [19] In 2-Chloro-diflurasone use as anti-inflammatory treatment containing methyl para toluene Sulphonate a potential genotoxic impurity developed. GIs derived from residual Starting raw material, solvent, reagent, Intermediate or side products formed during synthesis. The compound p-toluene sulfonic acid commonly used as catalyst or counter ion during synthesis its strong acidic and hydrophilic property it react with residual alcohols like methanol, ethanol during production or recrystallisation process during synthesis result in the formation of alkyl tosylate salt form with API having potential GTIs. These GTIs due to DNA alkylation produce mutagenicity or carcinogenicity.[20] Ethyl methane Sulphonate impurities presents in pharmaceutical products due to reaction between methane sulfonic acid and alcohols in synthesis of API.[21] Two unknown impurities (Impurity A & Impurity B) are developed in the synthesis of Rasagline which are second generation monoamine oxidase-B inhibitor are shown in Scheme: 2 and impurities are separated by HPLC& structure of impurities found by Mass spectroscopy &^{13}C-NMR technique. The structure of impurity A &B because the carbamates structures has been highlighted class of potential genotoxic impurities (GTIs) [22]



Scheme 2 : Synthetic scheme for Rasagline impurity A & B

Ziprasidone is a novel "atypical" or "second generation" antipsychotic drug. Orally administered, it is used for the treatment of schizophrenia. Ziprasidone and its one synthetic precursor (Impurity I) are photosensitive in solution due to isomerization of the benzisothiazole moiety to the corresponding benzthiazole. The mechanism of photoisomerization involves the azirine intermediate, and the respective structures were shown in Fig: 3& confirmed by means of LC/MS/MS and GC/MS. Impurities shown in Figure 3 such as, [23]

Impurity I: 3-(1-piperazinyl) -1,2-benzisothiazole

Impurity II: 6-chloro-5-(2-chloroethyl)-1,3dihydro-2H-indol-2-one

Impurity III: 5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]6-chloro-1,3-dihydro-2H-indol-2,3-dione

Impurity IV: 5,5′-bis[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl] ethyl]-6,6′-dichloro-1,1′,3,3′-tetrahydro-3-hydroxy[3,3′-bi-2H-indole]-2,2′-dione

Impurity V: 3-(1,2-benzisothiazol-3-yl)-5-[2-[4-(1,2-benzisothiazol3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2Hindol-2-one

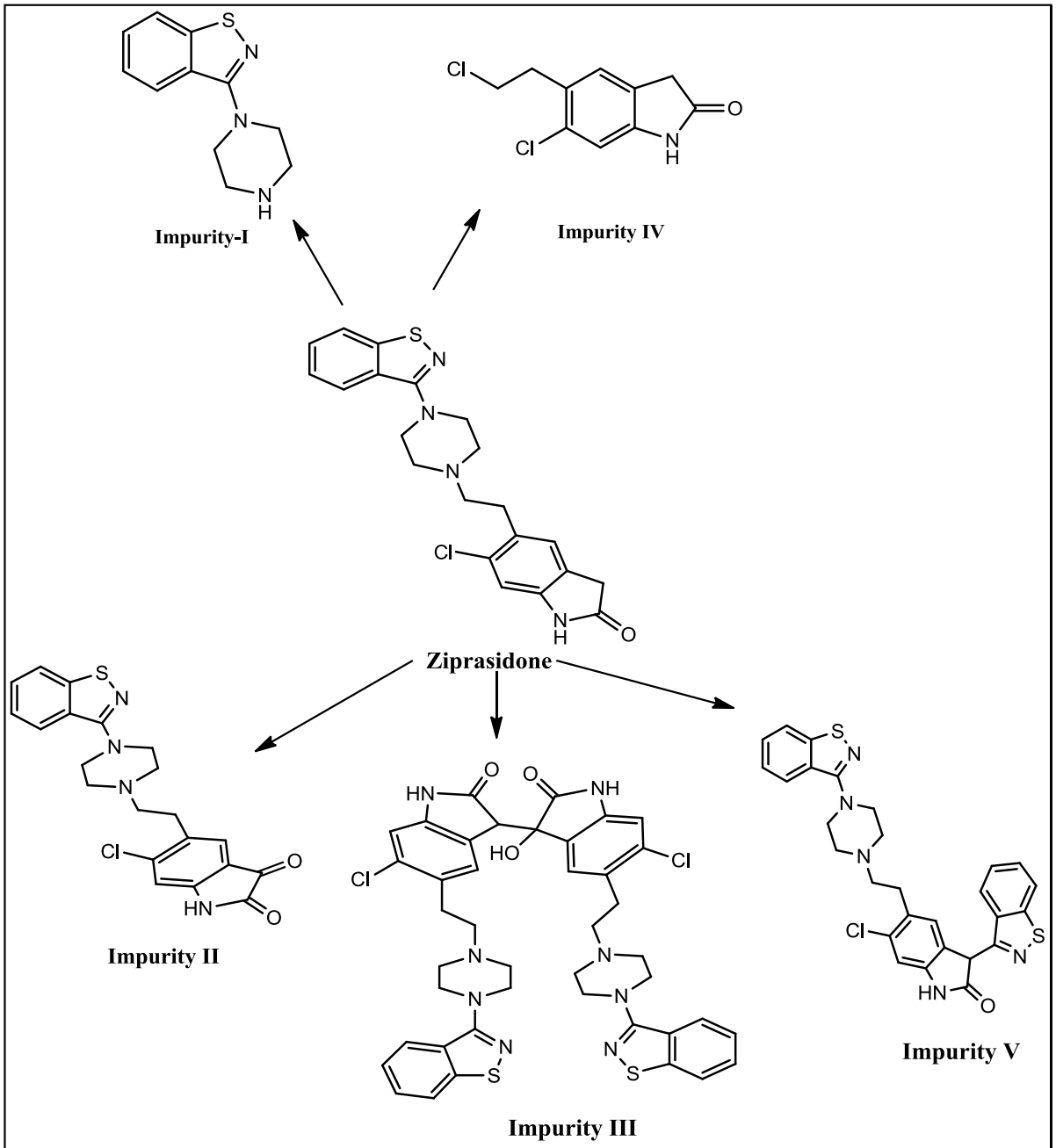
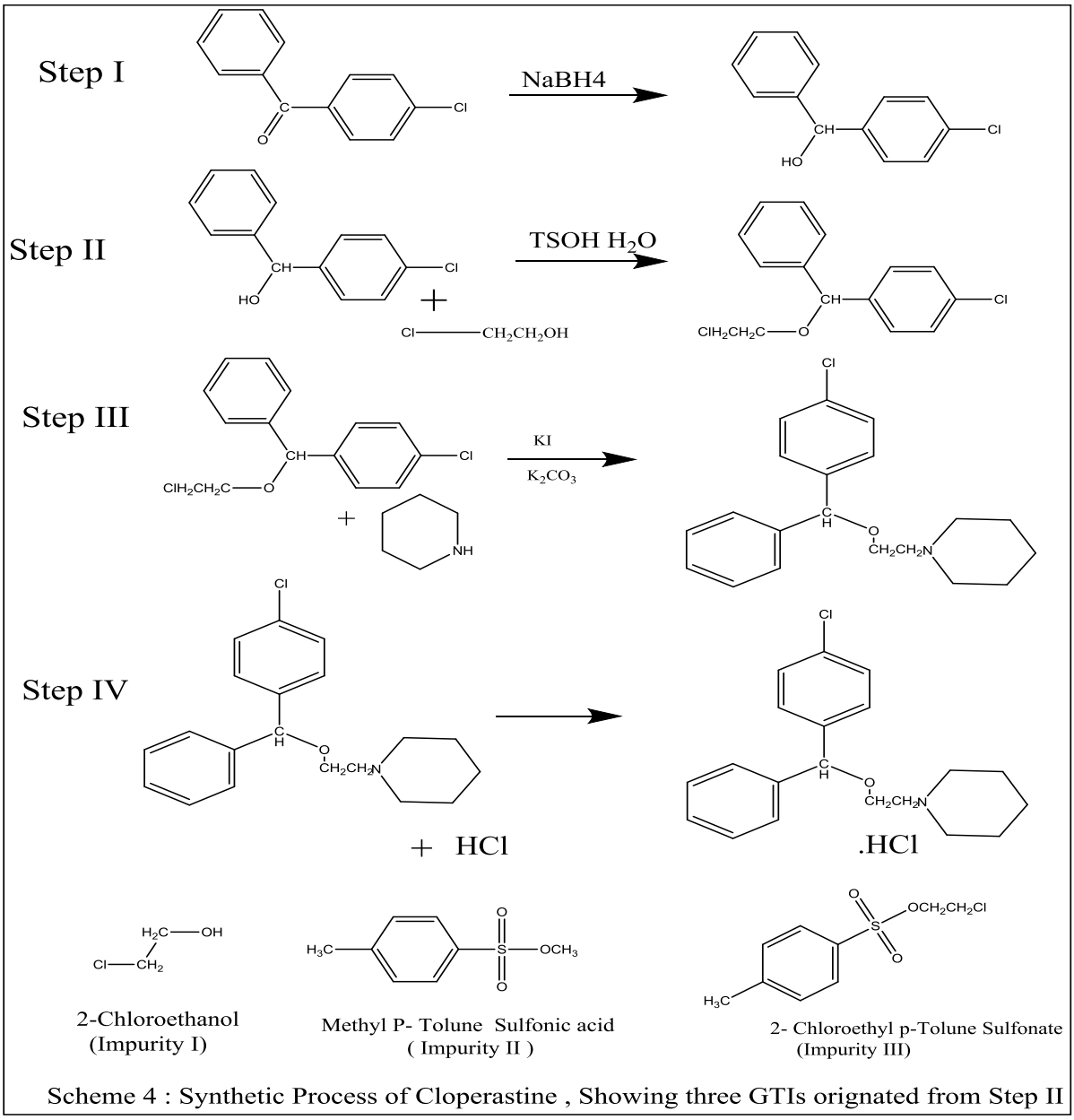


Fig 3: Ziprasidone & its Impurity

Cloperastine hydrochloride / fendizoate both are salt form act on cough center without depressing the respiratory center. Synthesis of Cloperastine consist four steps shown in scheme 4, At the end of reaction hydrochloride salt can be easily converted into fendizoate salt by the reaction with fendizoic acid. In the second step of synthesis p-Toluene sulfonic acid as a good leaving reagent, react with 2-chloroethanol and methanol (present in the reaction) can originate two alkyl esters of alkyl sulfonic acid as methyl p-Toluene sulfonic acid and 2-chloroethyl p-Toluene sulfonic acid having potent

genotoxicity shown in scheme 4 [25] These GTIs induce genetic mutation, chromosomal rearrangement or break cause a cancer.



Scheme 4 : Synthetic Process of Cloperastine , Showing three GTIs orignated from Step II

Table 5: HPLC methods for estimation of sulfonate esters

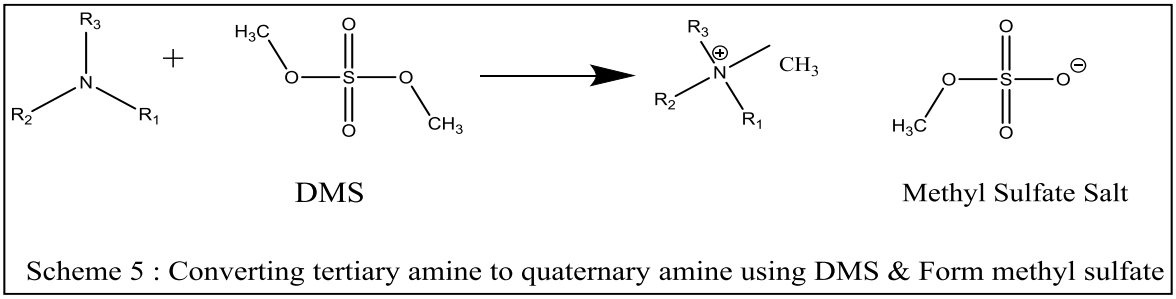
Sr. No	Drug	Impurity	Mobile Phase Composition	Mode of analysis	Column type	Column specification	Column temp.	Flow Rate	Detector	Detection wavelength	Retention time	Linearity	R2 value	Ref
1	Amlodipine Besylate	Methane sulfonate, Benzene sulfonate, and p-toluene sulfonate	Water: acetonitrile	Gradient elution	Kromasil C18 column	(250mm×4.6mm,5µm)	30 ^{0}C	1.0mL/ min	UV detector	-	3.3 min	15-150 ng/ml	0.998	11
2	Amlodipine Besylate	Methyl, ethyl isopropyl, n-propyl benzene Sulphonate	1% Triethylamine pH adjusted 3.0 with orthophosphoric acid: Acetonitrile (60:40 v/v)	Isocratic elusion	Inertsil ODS 3V Column C18 Column	(150mm×4.6mm,5µm)	Room temperature	1.0mL/ min	PDA detector	220 nm	-	75-180 µg/ml	0.999	12
3	ImatinibMesylate	Methyl methane Sulphonate	Solvents A: 2.8 gm of disodium hydrogen phosphate in 1000 mL with its pH adjusted to 2.70 with orthophosphoric acid Solvent B: consisted of buffer and mixture of water and acetonitrile in ratios of 10:90 (v/v)	Gradient elution	Develosil C8 UG-5 column	(150mm×4.6mm,5µm)	30 ^{0}C	0.5 mL/min	Visible wavelength detector	240 nm	4.3 & 16.7 min	3-15 µg/ml	0.998	14
4	ImatinibMesylate	Methyl methane Sulphonate	mobile phase A: 0.1% formic acid in 1000 mL of water was used as buffer mobile phase B.: acetonitrile	Gradient elution	Inertsil ODS 3V C18 Column	(150mm×4.6mm,5µm)	40 ^{0}C	1.0mL/ min	PDA detector	230 nm	-	1-30 ng/ml	0.999	15

5	ImatinibMesylate	Methyl methane Sulphonate	(phase A) ammonium formate 0.063% and (phase B) acetonitrile plus 0.05% formic acid	Gradient elution	Acquity BEH C18	(150 x 2.1 mm, 1.7 um)	40 ^{0}C	0.2 mL/min	UV/VIS detector	-	3.7 min	1.0 -5.0 µg/mL	0.998	16
6	Lopinavir	Methyl ethane sulfonate & Ethyl methane sulfonate	0.1% Formic acid (v/v): Acetonitrile	Gradient elution	Atlantis T3	(150 x 4.6mm, 3.0 um)	30 ^{0}C	1.0 mL/min	Flame Ionization detector	-	-	0.01-0.23 µg/mL	0.99	19
7	21-chloro-diflurasone	Methyl para toluene Sulphonate	A: 10mM Ammonium formate B: Acetonitrile (80:20 v/v)	Gradient elution	RP-Supelco discovery C-18 Column	(50 x 4.6mm, 5.0 um)	Room temp	1.0 mL/min	PDA detector	224 nm	-	-	-	20
8	Nelfinavir mesylate	Ethyl methane sulfonate	Acetonitrile: 100mM Ammonium acetate (93:7 v/v)	Isocratic elusion	Atlantis HILIC silica	(100 x 4.6mm, 3.0 um)	-	0.4 mL/min	Triple quadrupole mass detector	-	-	0-100 ng/ml	0.99	21
9	Rasagline mesylate	Methyl methane Sulphonate	Acetonitrile: water with 0.1% formic acid (1:1 v/v)	Gradient elution	Agilent poroshell 120 EC-C18	(50 x 4.6mm, 2.7 um)	30 ^{0}C	0.3 mL/min	PDA detector	215 nm	3.23 min	0.20-20 ng/ml	0.998	22
10	Ziprasidone mesylate	Impurity I, II, III, IV, V	A [buffer–acetonitrile (80 + 20, v/v)] and mobile phase B [buffer–acetonitrile (10 + 90, v/v)]	Isocratic elusion	Spherisorb octadecyl silyl 1 column	(250mm×4.6mm,5µm)	25 ^{0}C	1.5 mL/min	PDA detector	250 nm	7.9 min	70-130 µg/mL	0.999	23
11	Ziprasidone mesylate	Impurity I, II, III, IV, V	ammonium-formate buffer (10mM; pH 4.7) and acetonitrile as mobile phase,	Gradient elution	Acquity UPLC BEH Phenyl	(50x2.1 mm, 1.7µm)	30 ^{0}C	0.3 mL/min	PDA detector	-	-	-	-	24

12	Cloperastine fendizoate	Methyl p-toluene sulfonate & 2 Chloroethyl p-toluene sulfonate	3.0 pH phosphate buffer: methanol (contain 10% ACN) (45:55 v/v)	Isocratic elusion	Symmetry shield RP- 8 Column	(250mm×4.6mm,5µm)	-	1..7 mL/min	DAD detector	227 nm	-	-	-	25
16	-	Alkyl sulfonate and dialkyl sulfate	A: Acetonitrile B: 50mM ammonium formate with 0.1% formic acid (85:15 v/v)	Isocratic elusion	Atlantis HILIC silica	(50mm ×2.1 mm,3µm)	35 ^{0}C	0.3 mL/min	Mass detector	-	-	0.2-20 µg/mL	0.99	5
17	-	4-Flurobenzyl chloride	2.8 pH buffer: isopropanol: acetonitrile (7:5:88 v/v/v)	Isocratic elusion	Water Xbridge HILIC column	(100mm ×3.0 mm,3.5 µm)	-	0.5 mL/min	Mass /UV detector	290 nm	-	-	-	6
18	Lamivudine	Methane sulfonate & para toluene Sulphonate	A)0.01 M Ammonium acetate buffer: methanol (75:25 v/v) B) 0.01 M Ammonium acetate buffer: methanol (5:95 v/v)	Gradient elution	Zorbax Rx C8 Column	(250mm×4.6mm,5µm)	50 ^{0}C	1.0 mL/min	Mass detector	-	-	1.5-6.0 µg/mL	0.99	27

Gas Chromatography

Several drugs like imatinib mesylate containing methyl methane Sulphonate (MMS) and ethyl ethane Sulphonate (EMS) are produce as by products when methanol and ethanol are present in reaction matrix.The alkylation of MMS and EMS in Imatinib with DNA may cause cancer; these compounds are producing genotoxicity TTC level of these genotoxin 1.5 µg/day as per USFDA and EMEA guideline The LOD and LOQ were 0.1 and 0.5 µg/g of Imatinib mesylate respectively. RSD of MMS & EMS in imatinib mesylate injection was found to be 1.96 & 1.87 respectively.[17] Dimethyl sulfate (DMS) is one of the alkylating agents use in the organic synthesis having carcinogenicity. In these method DMS present in pantoprazole sodium injection. DMS use for the converting tertiary amine to quaternary amine and form methyl sulfate salt as intermediate shown in Scheme 5.[26]



Scheme 5 : Converting tertiary amine to quaternary amine using DMS & Form methyl sulfate

Table 6 : Gas-Chromatography methods for estimation of Sulfonate esters

Sr. no	Drug	Impurity	Column	Column Dimension	Carrier gas	Detector	Retention time	Ref
1	Doxazosin Mesylate	Methyl methane sulfonate	DB-5 (capillary column	30 m×0.32 mm×1.0 µm)	Helium was used as carrier gas at flow rate 1.46 ml/min	Mass detector	-	13
2	Imatinib Mesylate	Methyl methane sulfonate	HP-5MS column	(30 m × 0.32 mm × 0.25 µm)	Helium was used as carrier gas at a constant flow of 1 mL/min	Quadrupole mass analyzer	4.70 min	17
3	Imatinib Mesylate	Ethyl methane sulfonate	HP-5MS column	(30 m × 0.32 mm × 0.25 µm)	Helium was used as carrier gas at a constant flow of 1 mL/min	Quadrupole mass analyzer	5.13 min	17
4	-	Ethyl methane sulfonate Methyl methane sulfonate	DB-624 DB-1701 HP-5 MS UI column	(30m × 0.32mm × 1.8µm) (30m × 0.25mm × 0.25µm) (30 m × 0.25 mm × 0.25 µm),	Helium was used as the carrier gas at a constant flow of 1.2 mL/min	flame ionization detector (FID).	-	18
5	Cloperastine fendizoate	Methyl p-toluene sulfonate & 2 Chloroethyl p-toluene sulfonate Chloroethyl p-toluene sulfonate	VF-23ms Capillary column	(30 m × 0.25 mm × 0.25 µm),	Helium was used as the carrier gas at a constant flow of 0.8 mL/min	5975C single quadrupole mass spectrophotometer	19.0 min	25
6	Pantoprazole sodium	Dimethyl sulphate	A-DB 624 Capillary column	(30 m × 0.32 mm × 1.80 µm),	Helium was used as the carrier gas at a constant flow of 1.5 mL/min	Aglient 5973 mass selective detector	-	26
7	-	Alkyl mesylate and alkyl besylate	RistekRxi 5-sil MS capillary column	(30 m × 0.32 mm × 0.5 µm),	Helium was used as the carrier gas at a constant flow of 1.5 mL/min	Electron Impact detector	-	27
8	Lamivudine	Alkyl methane sulfonate and alkyl para toluene Sulphonate	GSBP-INOWAX Column	(30 m × 0.2 mm × 0.25 µm),	Helium was used as the carrier gas at a constant flow of 1.46 mL/min	Mass detector	-	28

CONCLUSION:

This reviews highlights the analysis of the various genotoxic impurity commonly found like Methyl ethane sulfonate, Ethyl methane sulfonate, Methyl p-toluene sulphonate, Dimethyl sulfonate. Chromatographic methods are mostly preferred for detection and analysis of genotoxic impurity by HPLC and GC. In HPLC reversed phase C18 column are used and detection with UV-visible and PDA detector. Gas chromatography was also a method of choice used for separation and detection of various genotoxic impurities separated by capillary column and detected by mass and Flame ionization detector..

In the present review various approaches of separation of alkyl and aryl sulfonate ester as a potent genotoxic impurity present in various active pharmaceutical ingredients (API) and its formulation are summarized.

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