ejpmr, 2020,7(4), 190-197

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

ANALYTICAL METHODS FOR THE DETERMINATION OF ANTICANCER DRUG PROCARBAZINE

***Nowreena K., Shiji Kumar P. S.[1], Sirajudheen M. K.[2] and Sherin A.**

[1]Department of Pharmaceutical Analysis, Jamia Salafiya Pharmacy College, Malappuram, India-673637.
[2]Department of Pharmaceutics, Jamia Salafiya Pharmacy College, Malappuram, India 673637.

***Corresponding Author: Nowreena K.**

Department of Pharmaceutical Analysis, Jamia Salafiya Pharmacy College, Malappuram, India-673637.

Article Received on 06/02/2020 **Article Revised on 26/02/2020** **Article Accepted on 16/03/2020**

ABSTRACT

Today the importance of the drugs for the treatments are very valuable, the number of patients receiving chemotherapy for cancer treatment. The cytotoxic drugs are mainly used for chemotherapy. Here procarbazine is a cytotoxic moiety and the action against cancer. The development of reliable analytical methods to analyze became necessary for treatments. From the discovery of new substances to patient administration, all types of analysis must verify the drug action in all direction. In this review, the various methods for the analysis of procarbazine and the its major metabolic products, is discussed.

KEYWORDS: Procarbazine, Metabolic products, Analytical methods.

INTRODUCTION

Cancer refers to one of the large number of diseases characterized by the development of abnormal cells that divide uncontrollably and have the ability to infiltrate and destroy normal body tissue. Cancer often has the ability to spread throughout the body. In the past, it has been one of the most common causes of death worldwide, with the most important treatments including surgery, chemotherapy or radiotherapy.[1]

Anti-cancer drugs can be classified according to their mechanism of action such as alkylation agents, antimetabolites, vinca alkaloids, taxanes, epipodophyllotoxins, camptothecin analogues, antibiotics, hormones, and other medications.[2]

Alkylation agents are compounds that produce highly reactive carbonium-ion intermediates that transfer alkyl groups to cellular macromolecules by forming covalent bonds. For example: dacarbazine, procarbazine and temozolomide.[3]

Today, with the increase in cancer patients treating with cytotoxic agents are widespread due to the aging population and the introduction of new treatments, treatment regimens will continue to be associated with cytotoxic drugs.

Procarbazine is significantly active against lymphomas and carcinomas of bronchus and brain tumors. The main side effects are observed as nausea, myelosuppression and hypersensitivity rash, which prevents further use of the drugs.

Consequently, the need for analytical methods for the determination of cancer drugs is of the utmost importance. The first method developed for the analysis of cytotoxic compounds are based on the use of liquid chromatography with UV detection (LC-UV).

These methods showed satisfactory quantitative performance for the analysis of samples containing high concentrations of target drugs (i.e. development of pharmaceutical formulations, stability studies, etc.). However, for samples containing a small amount of cytotoxins (i.e. biological or environmental analyses), a sample preparation step had to be applied prior to the LC-UV analysis, which enabled a pre- concentration of target compounds. In the 1990s, the high selectivity and sensitivity of mass spectrometry revolutionized the entire analysis process by simplifying and reducing the sample preparation step. Today, LC-MS is undoubtedly one of the techniques of choice for the analysis of cancer drugs with very attractive analytical performance. The detection limit (LOD) in the order $ngmL^{-1}$ is often reached. Other detections are also available.

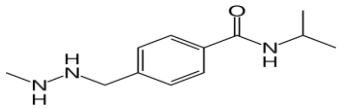
Procarbazine have analytical studies with human plasma by liquid chromatography with electrospray ionization mass spectrometry, the metabolites of drug analysis by gas chromatography mass spectrometry (GS-MS), High-performance liquid chromatography (HPLC) analysis of procarbazine hydrochloride by application of an electrochemical detector, pharmaceutical studies on procarbazine and its metabolites, in the spectral analysis of procarbazine mainly by normal coordinate spectra with spectra for structural determination, separation

techniques also in the dosage form of procarbazine, SPE and UPLC-ESI MS-MS of procarbazine in waste water, use UV-Visible spectroscopy with calf thymus DNA, UV spectroscopy and analysis OF procarbazine decomposition to their metabolites.

Procarbazine, N-isopropyl--(2-methylhydrazino)- p-toluamide, its pharmacokinetic behavior remains poorly understood, both in laboratory animals and in humans, although more than three decades have passed since the drug approved for clinical use for the first time. This may be a consequence of difficulties associated with the development of sufficiently sensitive and specific analytical methods to measure the concentration of procarbazine in biological fluids during the period during which the medicinal product is preclinical. and clinical development. As with other alkylation agents, the apparent instability of procarbazine in aqueous solution was an important concern for an assay suitable for use in pharmacokinetic studies.[4]

The antineoplastic activity of procarbazine results from its conversion into highly reactive alkylation species by cyp-450 mediated hepatic oxidative metabolism. Supportive medications. Supportive drugs, which are commonly used in the clinical treatment of patients with brain tumors, such as some anti-seizure drugs and corticosteroids, have been shown to significantly increase the systemic clearance of many anti- cancer agents. Improve. In recognition of the potential for such a pharmacokinetic interaction with procarbazine, a phase I clinical trial was recently launched to determine the maximum tolerated dose of the drug in patients with advanced glioma, at the same time, they received enzyme-inducing antiseizure drugs (EIASDs). Clarifying the influence of various antiseizure drugs on the pharmacokinetic behavior of procarbazine was considered an important aspect of this study.[5]

STRUCTURE OF PROCARBAZINE



ANALYTICAL METHODS

1) Studies on the metabolic products by mass spectrometry

The properties of the drug were investigated using isotope labelling and high-resolution mass spectrometry. The mass spectra of procarbazine and three of its degradation products. ie, isopropyl-2-alpha(2methylhydrazino)-p- toluamide1, N-isopropyl-2-alpha-(-2-methylazo)- p-toluamide2, N-isopropyl-alpha(2methylhydrazono)-p-toluamide 3 and 4 formylbenzoic acid isopropylamide 4[6], were investigated with isotope Mass spectrometry and data from metastable transitions. The complete 70 eV mass spectra of 1-4 are performed in Figure (1-4). Partial mass spectra of the compounds indicating the mass displacements of the main peaks in the spectra of their respective marked analogues, a specific intramolecular methyl group transfer, induced by electron impact, was diagnostic used to switch between two isomeric derivatives. In vivo metabolites of drugs in rats are identified by mass spectral analysis.[7,8,25]

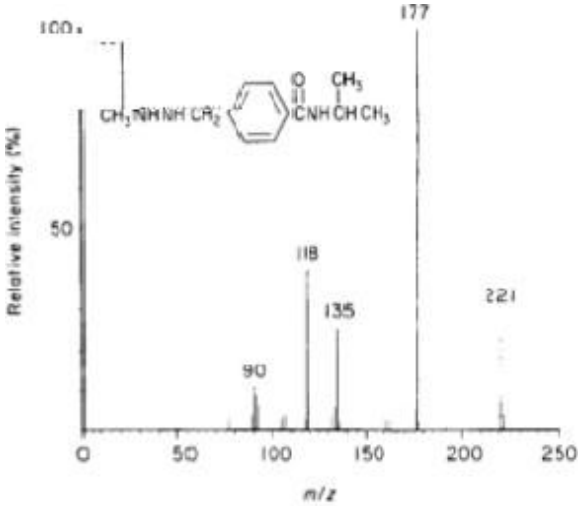


Figure 1. Mass spectrum (70 eV) of procarbazine (1).

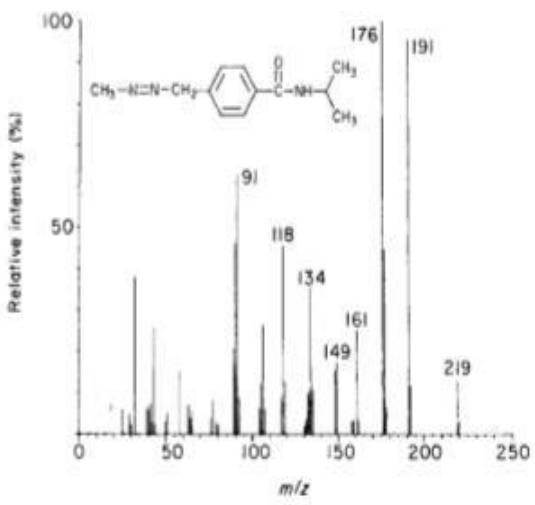


Figure 2. Mass spectrum (70 eV) of 2.

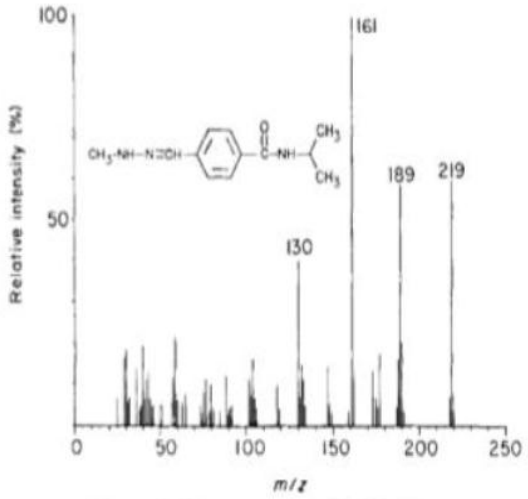


Figure 3. Mass spectrum (70 eV) of 3.

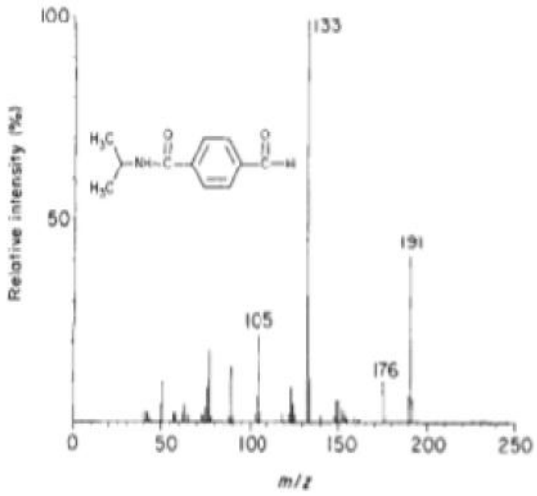


Figure 4. Mass spectrum (70 eV) of 4.

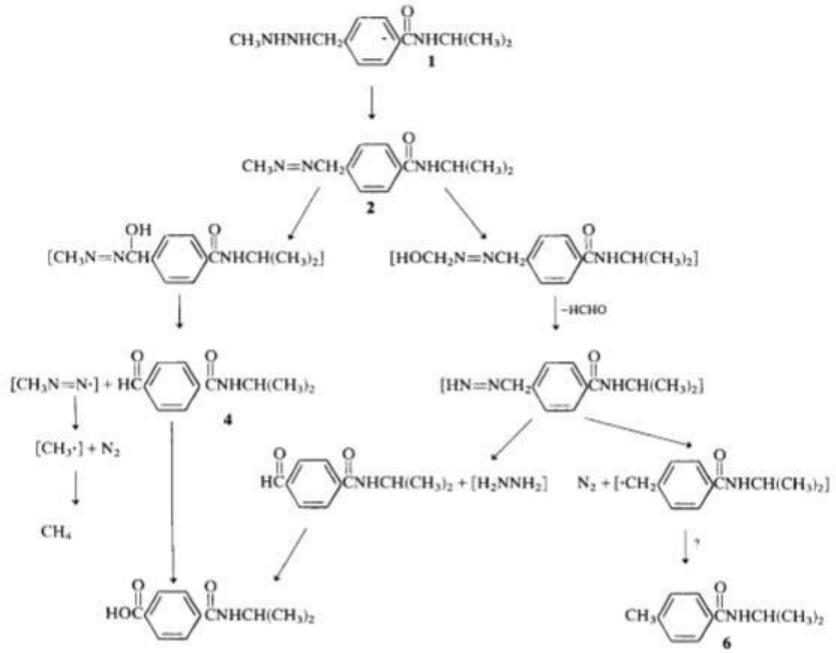


Figure 5: Metabilism of Procarbazine.

a) Metabolic studies

The metabolic pathway of procarbazine is severely impeded by the instability of both the parent drug and the proposed metabolites, which are shown schematically in Figure:5.

Rat plasma samples were analyzed using the same method to monitor possible interference. Coincidentally, there was a clean window at mass values that corresponded to the main diagnoses of the compounds of interest in the probe temperature range over which they eluted. Spectra were recorded at both 70 ev and 15 eV. There were no significant qualitative differences in the fragmentation patterns of compounds 1-4 in both energies. However, it was possible to obtain cleaner spectra of the biological samples after background subtraction with an ionizing energy of 15 eV. From the above the azo compound of procarbazine, N-isopropyl-2-alpha- (-2-methylazo)-p-toluamide was identified as the main circulating metabolite of the drug.

2) Gaschromatographyand mass spectrometry

Twelve compounds representing procarbazine, seven metabolites and an internal standard were analyzed by gas chromatography mass spectrometry on a 3% OV-1 column. Procarbazine and four metabolites were dissipated with acetic anhydride. A sensitive, specific and quantitative test was carried out by selected ion monitoring using a synthetic analogue of the drug as an internal standard. The detection limits were about 1 ng/ml plasma, while the limits of quantification were 10 ng/ml plasma. Studies on the degradation of procarbazine hydrochloride in 0.05 M phosphate buffer pH 7.4 were compared with in vivo studies. At 1 h after incubation of

procarbazine hydrochloride in the phosphate buffer, the azo and aldehyde metabolites were also detected as hydrazine, alcohol and N isopropyl-p-toluamide in the highest concentrations of 27.2% and 20.3% of the total drug and metabolites, as well as in small amounts of unchanged procarbazines. In the in vivo studies, analyses of rat plasmas showed that 1 h after an oral dose of procarbazine hydrochloride of aldehyde metabolite accounted for 72% of the total drug and metabolite and that relatively little of the azometabolite was present.[9]

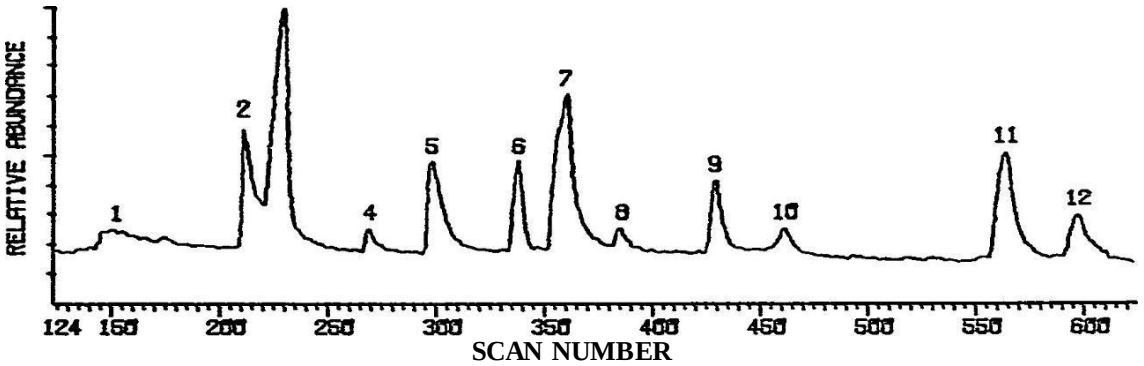


Figure 6: TLC plot of a mixture of procarbazine, metabolites, and degradation products after derivation with acetic anhydride.

The in vivo degradation and metabolism of the drug and its metabolites was even faster than the breakdown of the drug in the buffer. It is possible that the faster conversion of procarbazine in its metabolites in vivo is due to microsomal metabolism.[4] Since the content of the azo metabolite in the blood was significantly lower than that found in the buffer at a corresponding period, it seems likely that this metabolite is a major substrate for enzymatic metabolism.

The GC-MS assay described in this study provides a sensitive, rapid analysis of procarbazine and large metabolites. The data obtained from the animal study support the viability of this assay method for the determination of procarbazine and metabolites in biological systems.

3) Liquid chromatography with electrospray ionization mass spectrometry

The pharmacokinetics of procarbazine (N- isopropyl-alpha-(2-methylhydrazino)-p- toluamide) in brain tumor patients during a Phase I study, a method for determining the drug in human plasma by reverse phase high-performance liquid chromatography (HPLC) with electrospray ionization mass spectrometry (ESI-MS) was thoroughly validated. Plasma samples were prepared for analysis by precipitating proteins with trichloroacetic acid and washing the protein-free supernatant with methyl tert-butyl ether to remove excess acid. The solution was separated on a Luna C-18 analytical column with methanol 25mM ammonium acetate buffer, pH5.1(22:78, v/v) as a mobile phase at 1.0ml/min. The flow from the analytical column was fed into the electrospray ionization chamber of the MSD without splitting. The operating parameters of the API-ES interface were as follows: nebulizer pressure, 40psi; Drying gas (N2) Flow rate, 12l/min; Drying gas temperature, c; capillary stress, 1100V; Fragmentor voltage, 120V. The MSD was operated in positive ionization mode with selected ion monitoring (SIM) at m/z. (mass resolution, low). The duration for each chromatogram was 13.0min and the time between successive injections was about 14.5min.[10] Chromatograms have been integrated to provide peak areas with the data analysis capabilities of Agilent Chem Station Software (Rev. A.08.03).

A single-quadrupole mass spectrometer with electrospray interface was operated in the selected ion monitoring mode to detect the [M + H]+ ions at m/z 222.2 for procarbazine and at m/z 192.1 for the internal standard (3-dimethylamino- 2-methylpropiophenone) as an accurate and very sensitive detection method for the quantitative analysis of procarbazine in plasma. Procarbazine and the internal standard elutes as sharp, symmetrical peaks with retention times (mean s.D.) of 6.3 x 0.1 and 9.9 x 0.3min respectively. Calibration curves of procarbazine hydrochloride in human plasma in concentrations of 0.5 to 50ng/ml showed excellent linearity. The average absolute recovery of the drug from plasma was 102.9 to 1.0%. With a sample volume of 150l, procarbazine was determined at the lower quantitation limit of 0.5 ng/ml (1.9 nM) with an average accuracy of 105.2% and an interday precision of 3.60% R.S.D. on 11 different days over 5 weeks. During this time interval, the accuracy between the days for the determination of quality control solutions of the drug in plasma in concentrations of 2.0, 15 and 40ng/ml was between 97.5 and 98.2% (mean s.D., 97.9 x 0.4%) and the accuracy was 3.8-6.2% (mean s.d., 5.1 x 1.2%). Stability characteristics of the procarbazine were thoroughly evaluated to establish appropriate conditions to process, store and prepare clinical specimens for chromatographic analysis without inducing significant chemical degradation. The sensitivity achieved with this assay permitted the plasma concentration–time profile of the parent drug to be accurately defined following oral administration of standard doses to brain cancer patients.

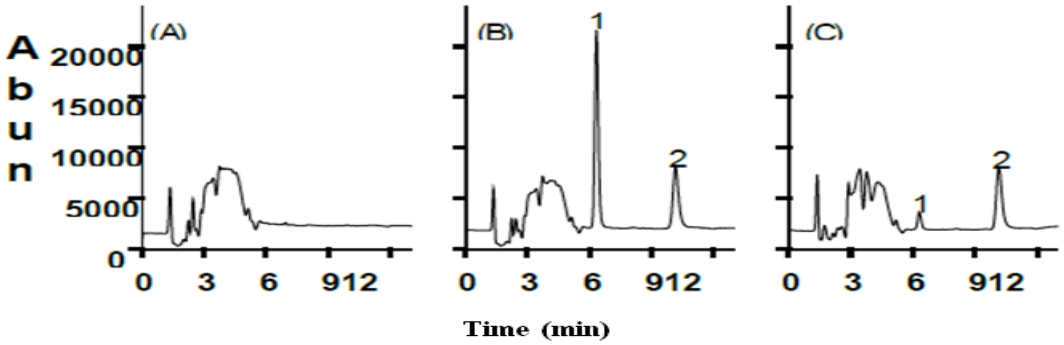


Figure 7:

Time-selected ion profiles depicting the abundance of positive ions at m/z 222.2 from 0 to 7.5min and m/z 192.1 from 7.5 to 13min during the LC/ESI–MS analysis of plasma samples. (A) Chromatogram of pretreatment plasma obtained from a brain cancer patient assayed without addition of internal standard. Chromatograms of plasma samples obtained 40min (B) and 4h (C) after oral administration of 200mg/m2 procarbazine to the same patient. Procarbazine (1) elutes near 6.3min and the internal standard (2) is the peak at 9.9min. The sample in panel B was diluted 10-fold with drug-free plasma prior to analysis. The area of the chromatographic peak in panel B corresponds to a procarbazine hydrochloride concentration of 39.3ng/ml in plasma and the concentration calculated from the peak in panel C is 3.5ng/ml.

4) An Electrochemical Detector on HPLC of Procarbazine

An amperometric flow-through detector with a paste working electrode was utilized as a high- performance liquid chromatographic (HPLC) detector to determine procarbazine hydrochloride, an antineoplastic agent, in both buffer solution and biological fluids. The HPLC system included an amino-cyano stationary phase and an aqueous (pH 7)-methanolic mobile phase which enabled the separation of procarbazine from its only electroactive degradation product, N-isopropyl-a(2-methylhydrazono)-p-toluamide. Utilizing HPLC with electrochemical detection, amounts below 100 ng/10 m l can be precisely and routinely determined. The electrochemical detector, with an approximate limit of detection of 2 ng procarbazine was 20 times more sensitive to procarbazine than a typical UV detector. The low dead volume (l VI) and superior selectivity of the electrochemical detector enabled the HPLC determination of procarbazine in untreated human urine and plasma.[11]

Calibration curve indicating linearity of electrochemical detector response with procarbazine between 10 and 1000 ng injected. graphically represents below.

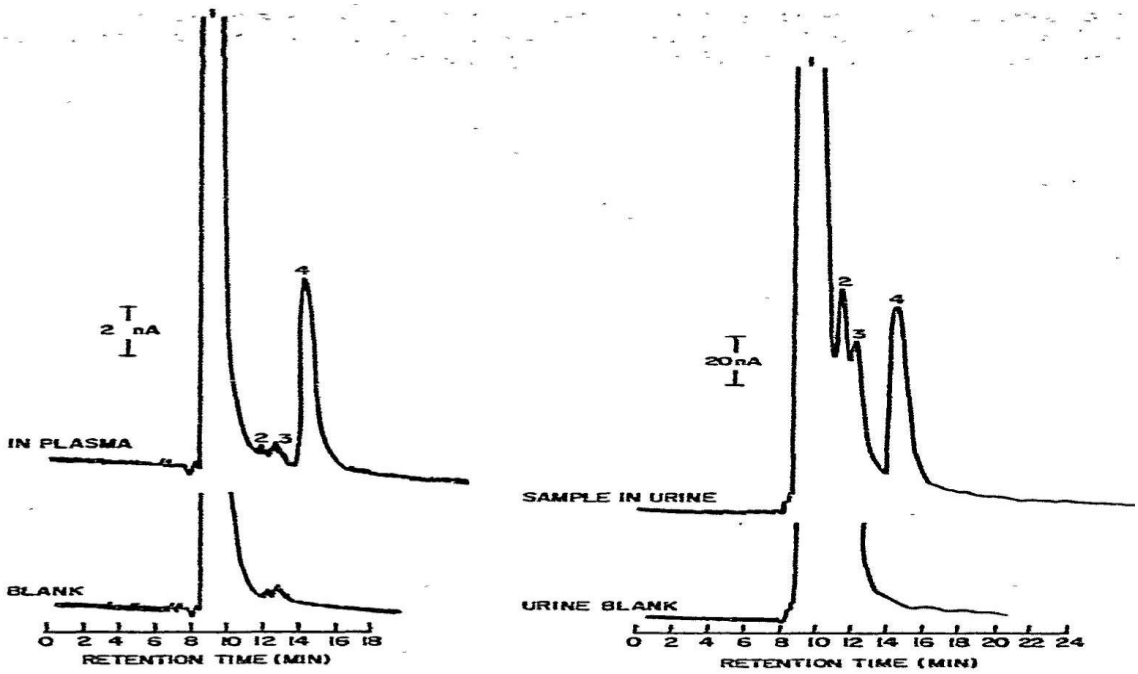


Figure 8: Electrochemical HPLC chromatogram of procarbazine in filtered human plasma and urine. (Peak; 1,2,3=plasma components,4=procarbazine & peak,1,2,3=urine,4=procarbazine) respectively.

5) Selective SPE & UPLC-MS-MS analysis in wastewater

Procarbazine in water samples from wastewater treatment plants has been developed a trace analysis method to study the cancer drugs. After concentration and cleaning with Oasis HLB solid phase extraction cartridges and Oasis WAX cartridges, the analytes were separated using ultra-high-performance liquid chromatography in conjunction with the electrospray ionization tandem compound. Spectrometry in positive ion mode.[12]

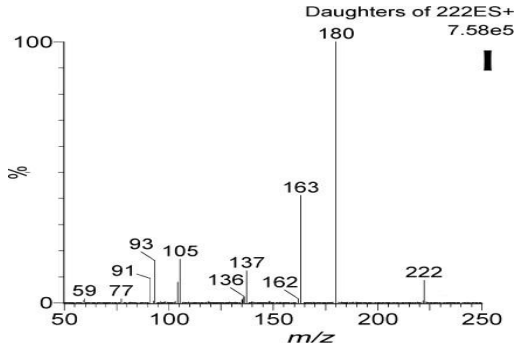


Figure 9: Typical collision-induced dissociation (CID) spectra for procarbazine.

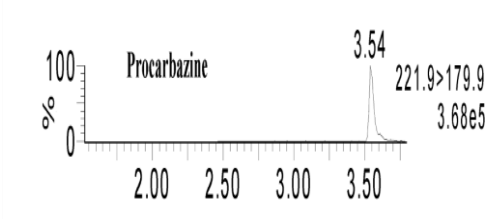


Figure 10: MRM spectra of procarbazine.

The process showed good linearity, precision and accuracy. The restoration of all analytes ranged from 45.3 to 108.9% with relative standard deviations of between 2.4 and 24.5%. The detection limits for wastewater and waste water were in the range of 0.6-7.0 ng/L and 0.5-3.5 ng/L respectively. The LOD of this method was approximately the same or less than previously reported methods. This method is expected to be used to investigate the ecological incidence of cancer drugs in wastewater.[13]

6) Vibration spectra and normal coordinate spectra on structure

Spectral analysis of drugs with high-grade pure sample of procarbazine was procured and used as such by VHB Laboratories, Mumbai. The FTIR spectrum of the connection was recorded in evacuation mode in evacuation mode with KBr pellet presses with 4.0 cm resolution. The FT Raman spectrum was recorded in flushing mode with a 200 mW YAG laser in flushing mode. The FTIR and FT-Raman spectra of procarbazine are shown below.[14]

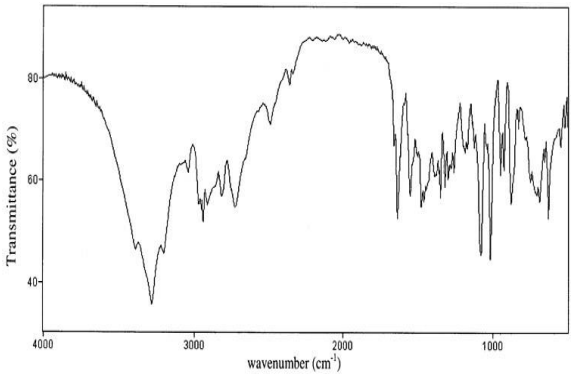


Figure 11: FTIR Spectra of Procarbazine.

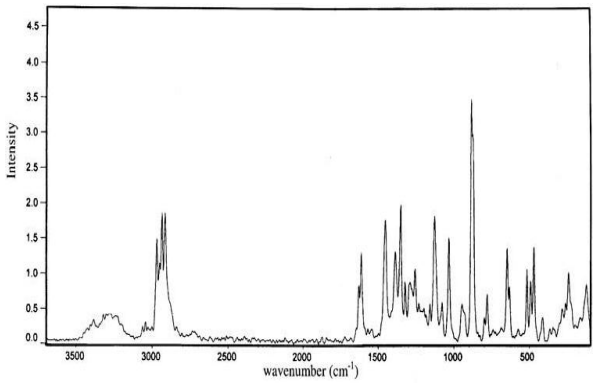


Figure 12: FT Raman spectra of Procarbazine

Interaction of drug with DNA can induce structural changes in the structure of DNA. With the help of CD spectroscopy, the change in the secondary structure of DNA in the presence of interacting drugs can be monitored.

7) UV-visible spectroscopy on a biophysical and molecular docking study of medicinal products

The interaction of procarbazine (PCZ) with calf thymus DNA was investigated using biophysical and molecular docking studies. Procarbazine should interact with DNA with a binding constant of 6.52 x 103 M 1 as calculated using ultraviolet- visible spectroscopy. To find out the binding mode, a molecular docking was performed, which PCZ predicted to interact with THE DNA (Calf Thymus DNA, CT DNA) through the groove binding mode with a binding affinity of 6.7 kcal/mol e. Various experiments were carried out to confirm the groove binding. Color shift tests confirmed non-intercalative binding mode. The interaction of PCZ with CT DNA was investigated with uv-visible spectroscopy. A fixed concentration of CT DNA (30 M) and titrated with increasing CONCENTRATION of PCZ. Spectra were scanned from 230 to 300 nm with a cuvette of 1 x 1 cm path length on shimadzu spectrophotometer (Japan). The basic correction was made with 10 mM Tris-HCl (pH 7.2).[15,16]

Procarbazine displaced Hoechst dye from the small groove of DNA, while it was unable to displace intercalating dyes. There was no increase in the viscosity of the DNA solution in the presence of PCZ. Also, a

negligible change in the secondary structure of DNA in the presence of PCZ was observed, as evident through circular dichroism spectra. Procarbazine may have caused a decrease in the melting temperature of DNA due to a decrease in the stability of DNA due to the creasing binding of PCZ with DNA.[17]

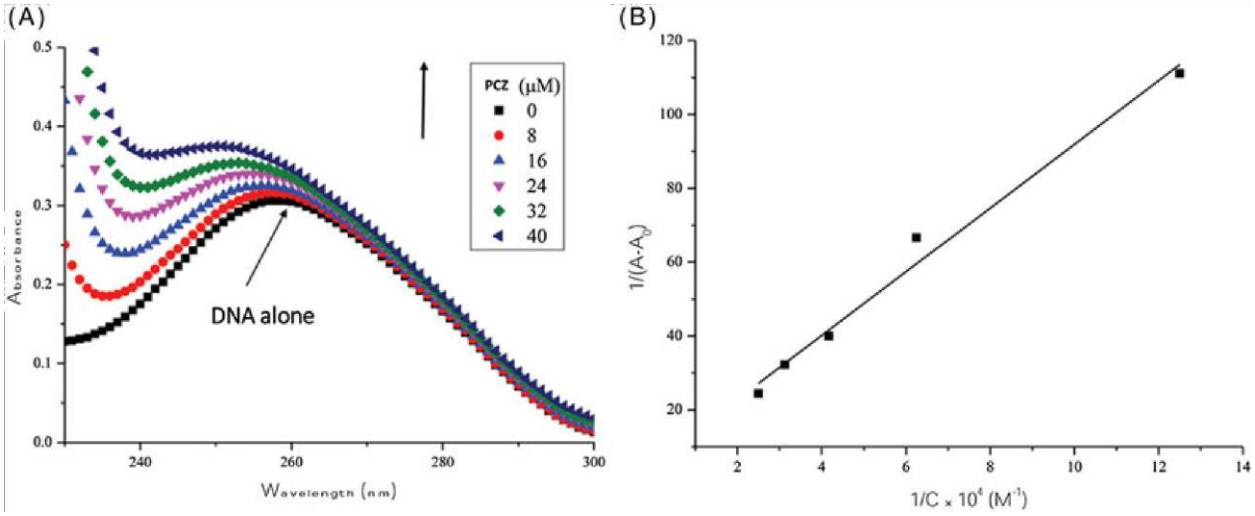


Figure 13:

Interaction of CT-DNA and procarbazine (PCZ) using Ultraviolet-visible spectroscopy. A, Ultraviolet-visible spectra of CT-DNA in presence of increasing concentration of PCZ (0-40 μM). B, Double reciprocal plot of 1/(A-A0) vs 1/C was obtained to calculate the binding constant

8) Separation and quantification of possible pharmaceutical products in their dosage form

With the help of high-pressure liquid chromatography, a stability-indicating assay for the degradation products of procarbazine hydrochloride was developed. The method uses a buffered methanol-water-mobile phase on an inverted phase column. Concentrations of degradation products of up to 0.04 mg/ml, 0.02% degradation, can be quantified with an internal standard of cinnamyl alcohol. Small amounts of degradation products were added to a sample of procarbazine hydrochloride in capsule material to test whether the extraction was complete and whether the presence of large quantities of procarbazine hydrochloride would affect the analysis of the degradation products.[18,19,20] The typical range for degradation products in procarbazine capsules is 0.1-0.5% after 4.5 years.

9) Determination of terephthalic acid isopropyl amide in urine by LC/MS

For the determination of terephthalic acid isopropylamide, the final metabolite procarbazine in human urine, a sensitive and simple liquid chromatography/mass spectrometry (LC/MS) was developed.[21] Fixed phase extraction with C18 cartridges was used, followed by LC/MS with a single mass spectrometer (SSQ 7000 from Finnigan). the quantification limit was 30 ng/ml in the urine (6 noises). A simple and sensitive LC/MS method has been developed to determine the most important procarbazine metabolite terephthalic acid isopropylamide in the urine. A fixed phase extraction with Baker C18 cartridges was used.[22,23]

This test was used for drug monitoring of terephthalic acid isopropylamide in the urine after oral administration of procarbazine in children and adolescents with Hodgkin's lymphoma.[24]

CONCLUSION

The importance of a drug for a particular disease or symptoms to treat, for the treatments, the drug must be introduced through the market. For the introduction of a drug that should be analyzed with different methods. This can be through clinical trials, analytical methods, etc. These analyses are carried out from biological fluids, bodily fluids and can be carried out from wastewater, etc.

Here we discussed the various analytical methods for the determination of cancer drug procarbazine. The anti-cancer drugs are now widely used because of the growing population with cancer. Therefore, chemotherapy is very important, therefore the drugs are also very important for administration for the patient.

It includes, studies on the metabolism of medicinal products by mass spectrometry, gas chromatography and mass spectrometry, liquid chromatography with electrospray ionization mass spectrometry, an electrochemical detector on HPLC from Procarbazine, selective SPE & UPLC-MS-MS analysis in wastewater, vibration spectra and normal coordinate spectra on structure, UV-visible spectroscopy on a biophysical and molecular docking study of pharmaceuticals, separation and quantification of possible pharmaceutical products in their dosage form and determination of terephthalic acid isopropylamide in the urine by LC/MS.

Today, with the advent of new chemotherapy treatments

(including biological agents, hormones, and molecular targeting agents), the development of useful analytical methods for preclinical and clinical trials, but also for the development of formulations containing these compounds, is required and represents the next challenge in the analysis of cancer drugs.

AKNOWLEDGEMENT
Nil.

CONFLICT OF INTEREST
Nil.

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