MAR Pharmacology and Toxicology

Research Article

Deterimination of Cyclobenzaprine and N-Desmethylcyclobenzaprine, Chlorpheniramine and It's Analog in Liver Tissues

Ahmed M.A. Shihata*

*Corresponding Author: **Ahmed M.A. Shihata**, Consultant Forensic Toxicology, Forensic Medicine Authority – Chemical Lab, Egypt.

Received Date: March 01, 2021

Publication Date: March 10, 2021

Abstract

Drugs are synthetic molecules that are meant to correct altered physiology. Unfortunately, their healing capability comes up with some side effects that can be lethal in some cases. Even more surprising is the fact that an overdose of these drugs can put life-threatening impacts. Among these dangerous drugs, those that act upon a muscle relaxant and antidepressant are posing the highest risk. One such class of drugs is called muscle relaxants. Many people experience muscle spasms, twitching, and other deformities. In these cases, muscle relaxants are mostly prescribed by physicians.

These drugs can be risky to some extent if misused. Their continuous use or an overdose can cause serious complications that could prove fatal.

The present study was carried out to investigate Cyclobenzaprine (CB) a muscle relaxant and antidepressant and its metabolite desmethyl cyclobenzaprine, chlorpheniramine and its analog, Chlorphenamine is an antihistamine used to treat the symptoms of allergic conditions

Samples preparation was done by liquid-liquid extraction and using GCMS, The cause of death was determined to be acute intoxication by the combined effects of the three drugs cyclobenzaprine and chlorphenamine and dexchlorpheniramine.

Keywords: *Cyclobenzaprine and Chlorpheniramine, GC-MS, human tissues and blood*

Introduction

Cyclobenzaprine is a tricyclic antidepressant derivative that relaxes the skeletal muscle and also a central nervous system depressant, and its efficacy may be related to its sedative effects. Cyclobenzaprine is used for the treatment of painful muscle spasms from acute muscle conditions. The exact mechanism of action of cyclobenzaprine has not been fully determined, but this drug seems to primarily act at the brain stem to reduce tonic somatic motor activity, influencing both gamma and alpha motor neurons leading to a reduction in muscle spasms. Cyclobenzaprine (CB) is a 5HT2 receptor antagonist; it relieves muscle spasm through action on the central nervous system at the brain stem, rather than targeting the peripheral nervous system or muscles themselves **(**1**)**. Cyclobenzaprine is a very strong basic compound (based on its pKa) **fig.(1)**3-(5*H*-Dibenzo[*a,d*]cyclohepten–5–ylidene)-*N, N*-dimethyl–1–propanamine; and a centrally acting muscle relaxant and related to tricyclic antidepressant. It is used in the treatment of the musculoskeletal condition associated with painful muscle spasms **(**2**)**.

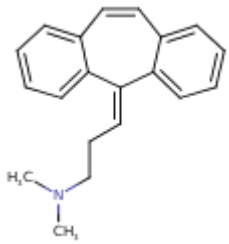

Figure 1. Chemical structure of cyclobenzaprine

It decreases pain in the first two weeks, peaking in the first few days, but has no proven benefit after two weeks **(**3,4,5**)**. Since no benefit is proven beyond that, therapy should not be continued long-term **(**6,7**)**.

The most common effects of overdose are drowsiness and tachycardia. Rare but potentially critical complications are cardiac arrest, abnormal heart rhythms, severe low blood pressure, seizures, and neuroleptic malignant syndrome, Life-threatening overdose is rare **(**6**)**, Cyclobenzaprine is extensively metabolized in the liver via both oxidative and conjugative pathways. It is structurally similar to Amitriptyline, differing by only one double bond. As the management of cyclobenzaprine overdose is complex and ever-changing, **fig. (2)**

N-Desmethylcyclobenzaprine chemical name 3-(5H-Dibenzo [a,d] cyclohepten-5-ylidene)-N-methyl-1-propanamine, or norcyclobenzaprine is the major urinary metabolite of the skeletal muscle relaxant cyclobenzaprine,

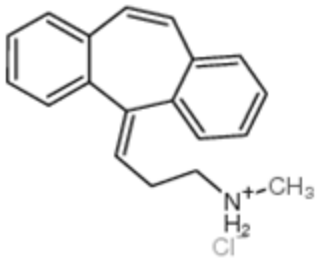

Figure 2. Chemical structure of desmethylcyclobenzaprine HCl

Chlorphenamine (CP, CPM)

Antihistamines are pharmaceutical agents which act by stimulating histamine action in the H1-receptors, thereby antagonizing most of the smooth muscles to alleviate or prevent the symptoms of hay fever and other allergies and put a stop to motion sickness, nausea, vomiting, and dizziness. In addition, since antihistamines may cause drowsiness as a side effect, some of them may be used as an opponent to insomnia. Some antihistamines are used in the handling of nervous and emotional conditions to help control anxiety and to relax patients before surgery **(**8**)**.

Chlorphenamine or Chlorpheniramine or Chlorprophenpyridamine (CP, CPM) **fig. (3**) is a first-generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria, It is worked by blocking the H1 receptor **(**9**)**. Chlorphenamine is a part of a series including pheniramine and its halogenated derivativesincluding fluorpheniramine, dexchlorphenamine, brompheniramine, dexbrompheniramine, deschlorpheniramine, and iodopheniramine. The halogenated alkylamine antihistamines all exhibit optical isomerism, and chlorphenamine in the indicated products is racemic chlorphenamine maleate, whereas dexchlorphenamine is the dextrorotary stereoisomer. These compounds belong to the class of organic compounds known as pheniramine and containing a pheniramine moiety, which is structurally characterized by the presence of a 2-benzylpyridine linked to an dimethyl(propyl)amine to form a dimethyl[3-phenyl-3-(pyridin-2-yl)propyl] amine skeleton.

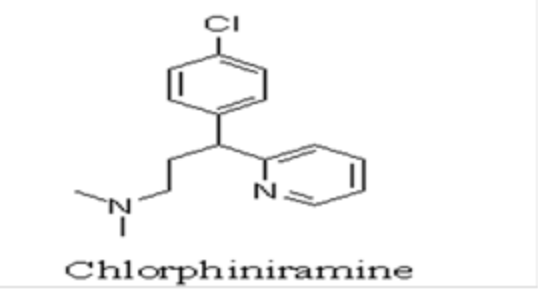

Figure 3. Chemical structure of chlorpheniramine

Dexchlorpheniramine is an antihistamine with anticholinergic properties used to treat allergic conditions such as hay fever or urticaria **(**11**)**. It is the pharmacologically active dextrorotatory isomer of chlorpheniramine.

Dexchlorphenamine had Ki values for the human cloned H1 receptor of 2.67 to 4.81 nM while levchlorphenamine had Ki values of 211 to 361 nM for this receptor, indicating that dexchlorphenamine is the active enantiomer **(**11**)**. Another study found that dexchlorphenamine had a Ki value of 20 to 30 μM for the muscarinic acetylcholine receptor using rat brain tissue while levchlorphenamine had a Ki value of 40 to 50 μM for this receptor, indicating that both enantiomers have very low affinity for it **(**12**)**.

Experimental procedure

Standard and reagents

Pure sample Cyclobenzaprine (CB) and chlorphenamine were kindly supplied by Multi-Apex Pharma, Cairo, Egypt. and the other chemicals were of analytical grade. Methanol HPLC grade and Ammonium sulfate, HCL, Ethyl acetate Algomhoria company Cairo - Egypt.

Sample preparations:

Liver specimens were collected at autopsy, Liver homogenates were prepared by adding saturated ammonium sulfate acidified by hydrochloric acid overnight then filtrate and converting alkaline medium by ammonia, extraction by ethyl acetate.

Chromatographic conditions

Gas chromatography(Agilent 5977) coupled to amass spectrometry, Column of GC-MS is Agilent HP-5-MS; 0.25mm x 30m x 0.25um.

Helium gas type and pressure: 20.969 psi and flow 1.5ml/min, the average velocity: 32 cm/sec. run in full-scan mode (scan range 50-550 *m/z*) with a solvent delay at 3.0 minutes.

Calibration work:

MassHunter creates a calibration curve by plotting response ratio vs. concentration. To determine the concentration of a sample, the response ratio is determined and the concentration can be calculated using the regression equation for the curve. Eight concentrations (0.1, 0.5, 10, 20, 50, 100 and 200 ug/mL) of the analytes were prepared and analyzed in three different analytical runs. The presence of quantifier ions m/z values and qualifier ions m/z values at their respective retention times was required to deem a calibration point usable for the determination of the calibration model and subsequent studies.

Limit of detection (LOD)

The LOD is an estimate of the lowest concentration of an analyte in a sample that is reliably differentiated from the signal due to the blank matrix and identified by the analytical method used, LOD may be estimated from a minimum of three linear calibration curves constructed over the working concentration range over different runs.

Precision: Precision is the closeness of agreement between a series of measurements obtained from multiple samplings of the same homogenous sample. The same data from the bias study was used to evaluate within-run and between-run precision.

Results and discussion

The screening of the postmortem liver specimen for pesticides and insecticides, other toxic compounds, opiates, benzoylecgonine, benzodiazepine, amphetamines, cannabinoids, propoxyphene, tramadol and other routine analyses was negative results. **Figs. (4,5)** The results in an efficient chromatography for

cyclobenzaprine retention time is 9.27 min while retention time of N-desmethyl cyclobenzaprine at 9.34, the main ions fragmentation of cyclobenzaprine are *m/z* 58, 59, 91, 189, 215.

The principle ions fragmentation of N-desmethyl cyclobenzaprine are *m/z* 55, 58, 91, 189, 215, 261. **Figs. (6,7)** chlorpheniramine retention time is 8.38 min, two methods of metabolites of chlorpheniramine are N-dealkylation oxidative deamination. The retention time of dexchlorheniramine at 8.37, the main ions fragmentation of chlorpheniramine are *m/z* 58, 77, 105, 129, 167, 185, 203. The principle ions fragmentation of dexchlorheniramine are *m/z* 58, 72, 91, 105, 129, 167, 181, 203.

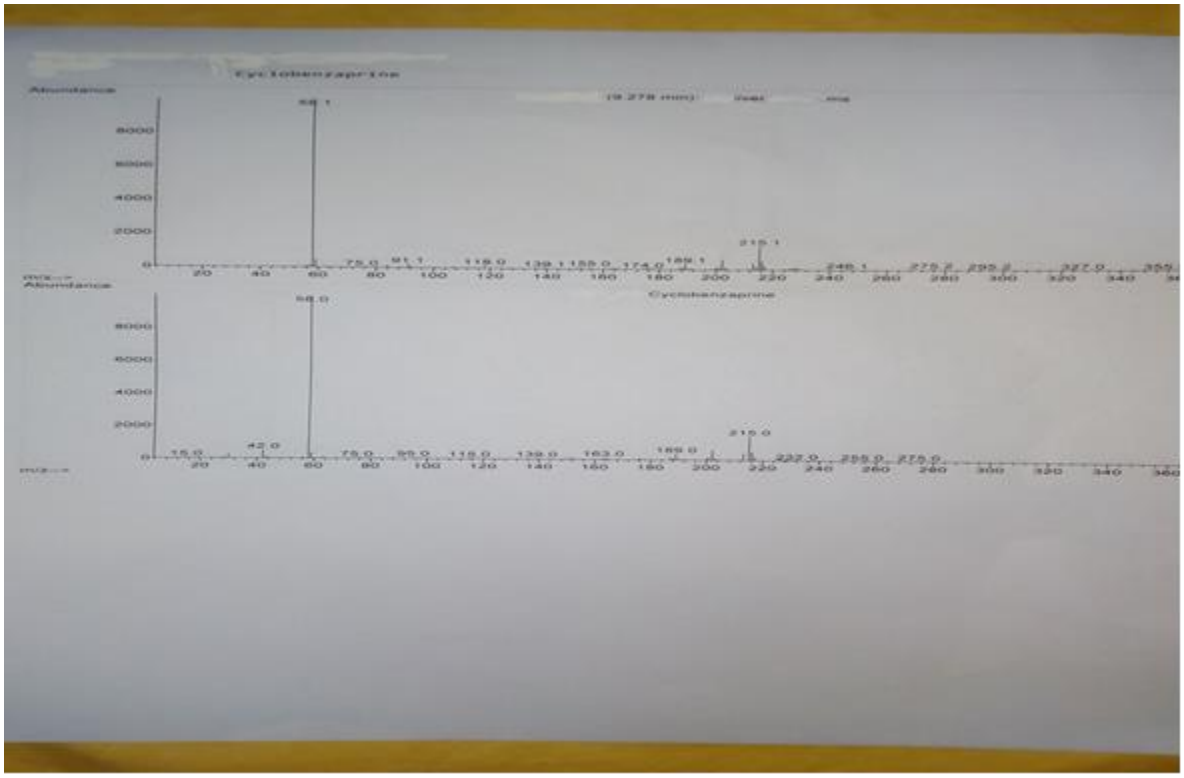

Figure 4 Cyclobenzaprine principal ions at m/z 58, 59, 91, 189, 215.

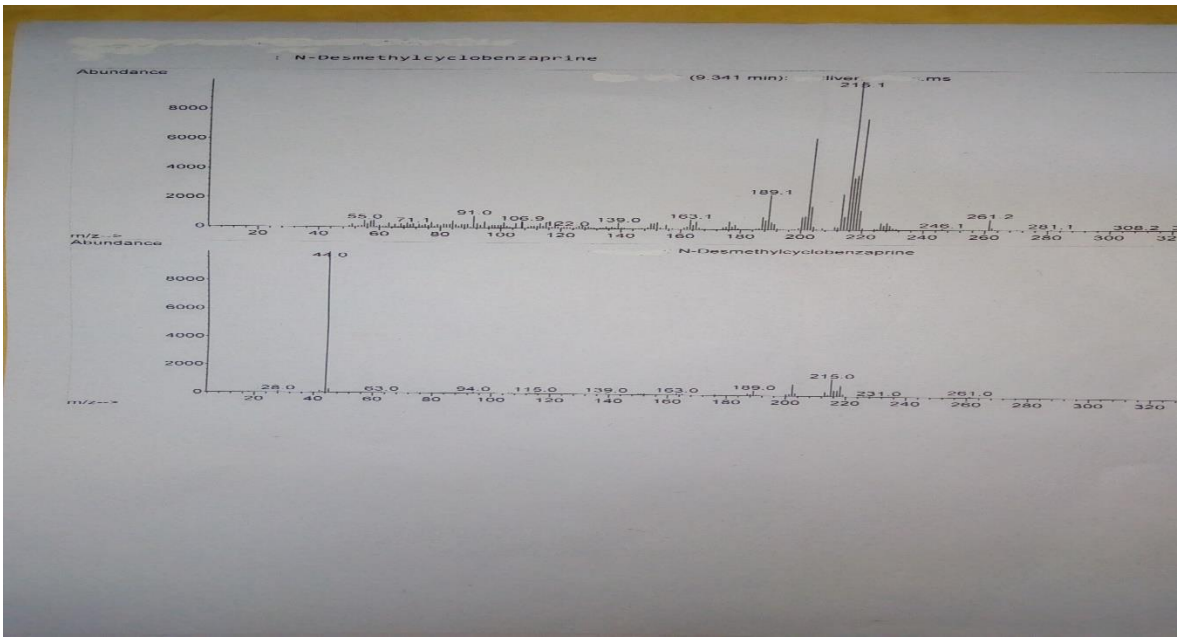

Figure 5 N-desmethyl cyclobenzaprine principal ions at m/z 55, 58, 91, 189, 215, 261

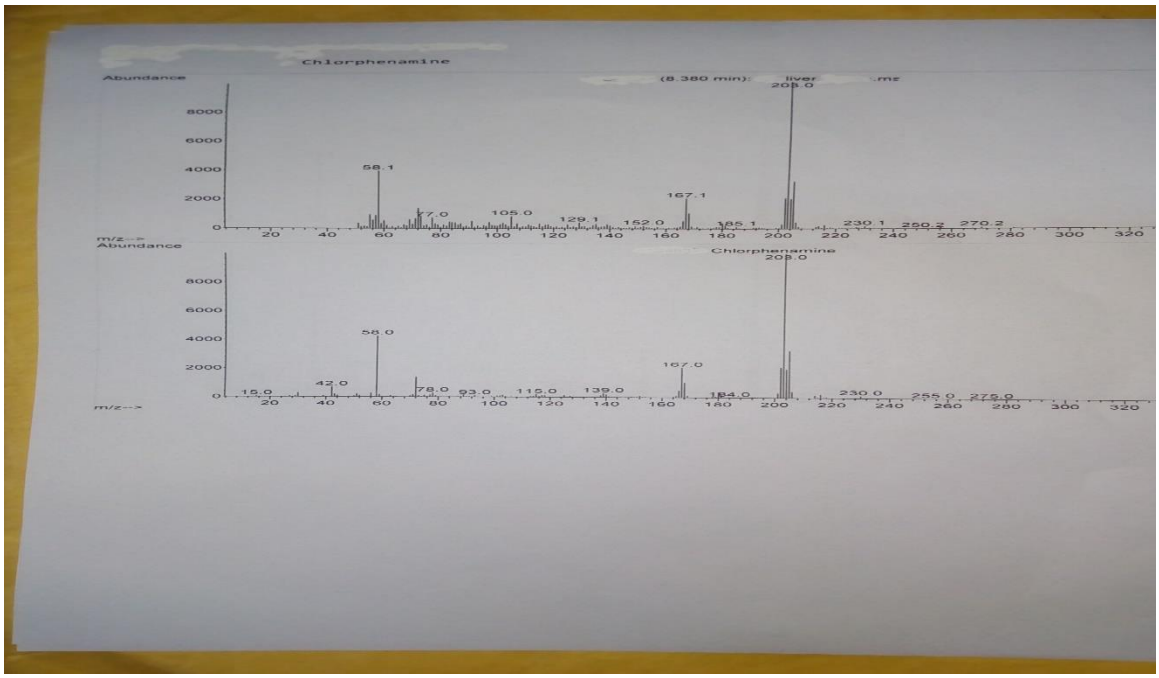

Figure 6 chlorpheniramine principal ions at m/z 58, 77, 105, 129, 167, 185, 203

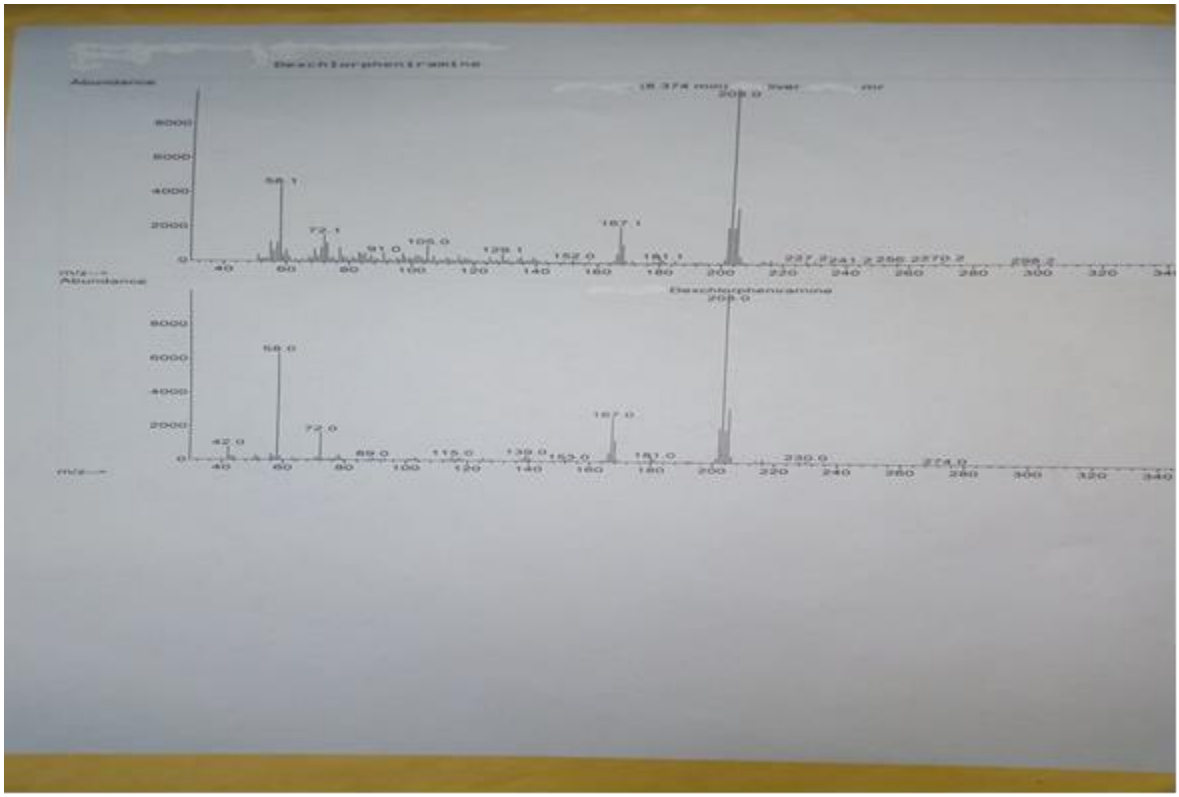

Figure 7 dexchlorheniramine principal ions at m/z 58, 72, 91, 105, 129, 167, 181, 203.

Toxicity cyclobenzaprine and chlorphenamine

A 40 years old man was found dead in his flat, cyclobenzaprine was the suspected cause of death, liver postmortem tissue concentrations liver 11.12 µg/g. Chlorphenamine concentration was found in liver tissues 1.5 mg/g. The cause of death was determined to be acute intoxication by the combined effects of the two drugs Toxicity cyclobenzaprine and chlorphenamine

Conclusion

These drugs can be risky to some extent if misused. Their continuous use or an overdose can cause serious complications that could prove fatal.

References

1- Kobayashi H, Hasegawa Y, Ono H (September 1996). "Cyclobenzaprine, a centrally acting muscle relaxant, acts on descending serotonergic systems". European Journal of Pharmacology. 311 (1): 29–35. doi:10.1016/0014-2999(96)00402-5. PMID 8884233.

2- Martindale (2002) The Complete Drug Reference. 3rd edn. Pharmaceutical Press, UK.

3- Chou R, Peterson K, Helfand M (August 2004). "Comparative efficacy and safety of skeletal muscle relaxants for spasticity and musculoskeletal conditions: a systematic review". Journal of Pain and Symptom Management. 28 (2):140–75. doi:10.1016/j.jpainsymman.2004.05.002. PMID 15276195.

4- van Tulder MW, Touray T, Furlan AD, Solway S, Bouter LM (2003). Van Tulder MW (ed.). "Muscle relaxants for non-specific low back pain". The Cochrane Database of Systematic Reviews. 2(2): CD004252. doi:10.1002/14651858.CD004252. PMC 6464310. PMID 12804507.

5- Browning R, Jackson JL, O'Malley PG (July 2001). "Cyclobenzaprine and back pain: a meta-analysis". Archives of Internal Medicine. 161 (13): 1613–20. doi:10.1001/archinte.161.13.1613.PMID 11434793.

6- "Cyclobenzaprine official FDA information, side effects, and uses". Drugs.com. October 2009. Retrieved 19 February 2010.

7- "Flexeril (Cyclobenzaprine HCl) Tablets". Food and Drug Administration. 2003. Retrieved 26 July 2009.

8- J. E. F. Reynolds, Martindale, The Extra Pharmacopoeia, The Pharmaceutical Press, London, 30th edn, 1993.

9- Yasuda SU, Wellstein A, Likhari P, Barbey JT, Woosley RL (1995). "Chlorpheniramine plasma concentration and histamine H1-receptor occupancy". Clin. Pharmacol. Ther. 58 (2): 210–20.doi:10.1016/0009-9236(95)90199-X. PMID 7648771.

10- Ortíz San Román, L.; Sanavia Morán, E.; Campos Domínguez, M.; Peinador García, M. M. (2013). "Síndrome anticolinérgico por dexclorfeniramina como causa de retención urinaria".Anales de Pediatría. 79 (6):4001. doi:10.1016/j.anpedi.2013.02.014. PMID 23680058.

11- Booth RG, Moniri NH, Bakker RA, Choksi NY, Nix WB, Timmerman H, Leurs R (2002). "A novel phenylaminotetralin radioligand reveals a subpopulation of histamine H(1) receptors". J. Pharmacol. Exp. Ther. 302 (1): 328–36. doi:10.1124/jpet.302.1.328. PMID 12065734.

12- Yamamura HI, Snyder SH (1974). "Muscarinic cholinergic binding in rat brain". Proc. Natl. Acad. Sci. U.S.A. 71 (5):1725 doi:10.1073/pnas.71.5.1725. PMC 388311. PMID 4151898.

Volume 2 Issue 3 April 2021